

JOURNAL OF GENETICS

CAMBRIDGE UNIVERSITY PRESS

C. F. CLAY, MANAGER

LONDON: FETTER LANE, E.C. 4



LONDON: H. K. LEWIS AND CO., LTD., 136 Gower Street, W.C. 1

LONDON: WILLIAM WESLEY AND SON, 28 Essex Street, Strand, W.C. 2

CHICAGO: THE UNIVERSITY OF CHICAGO PRESS

BOMBAY, CALCUTTA, MADRAS: MACMILLAN AND CO., LTD.

TORONTO: J. M. DENT AND SONS, LTD.

TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

All rights reserved

JOURNAL OF GENETICS

EDITED BY

W. BATESON, M.A., F.R.S.

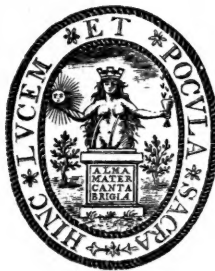
DIRECTOR OF THE JOHN INNES HORTICULTURAL INSTITUTION

AND

R. C. PUNNETT, M.A., F.R.S.

ARTHUR BALFOUR PROFESSOR OF GENETICS IN THE UNIVERSITY OF CAMBRIDGE

Volume IX. 1919-1920



168869.
24/1/22

Cambridge
at the University Press

1920

QH
431
A1J64
v. 9
cop. 2

CONTENTS

No. 1 (December, 1919)

	PAGE
J. W. HESLOP HARRISON. Studies in the Hybrid <i>Bistoninae</i> . IV. Concerning the Sex and Related Problems. (With Plate I and ten text-figures)	1
J. W. HESLOP HARRISON. A Preliminary Study of the Effects of Administering Ethyl Alcohol to the Lepidopterous Insect <i>Selenia bilunaria</i> , with Particular Reference to the Offspring . . .	39
H. ONSLOW. Inheritance of Wing Colour in Lepidoptera. II. Melanism in <i>Tephrosia consonaria</i> (var. <i>nigra</i> Bankes). (With Plate II) .	53
JOHS. SCHMIDT. Racial Studies in Fishes. III. Diallel Crossings with Trout (<i>Salmo trutta</i> L.)	61
C. J. BOND. On Certain Factors concerned in the production of Eye Colour in Birds	69
CHR. WRIEDT. The Brindle Colour in Cattle in Relation to Red . .	83

No. 2 (January, 1920)

A. B. STOUT. Further experimental studies on Self-incompatibility in Hermaphrodite Plants. (With Plates III, IV)	85
J. E. DUERDEN. Methods of Degeneration in the Ostrich. (With Plates V, VI and eight text-figures)	131

No. 3 (February, 1920)

J. W. HESLOP HARRISON. Genetical Studies in the Moths of the Geometrid Genus <i>Oporabia</i> (<i>Oporinia</i>) with a special consideration of Melanism in the Lepidoptera. (With thirteen text-figures)	195
FRANCES PITT. Notes on the Inheritance of Colour and Markings in Pedigree Hereford Cattle. (With Plates VII—X)	281

No. 4 (March, 1920)

	PAGE
R. C. PUNNETT. The Genetics of the Dutch Rabbit—A criticism. (With two text-figures and Plate XI)	303
MASAO SÔ and YOSHITAKA IMAI. The Types of Spotting in Mice and their Genetic behaviour. (With Plate XII)	319
L. DONCASTER. The Tortoiseshell Tomcat—A Suggestion.	335
H. ONSLOW. The Inheritance of Wing Colour in Lepidoptera. III. <i>Melanism in Boarmia consortaria</i> (var. <i>consobrinaria</i> , Bkh.). (With Plate XIII)	339
E. J. ALLEN and E. W. SEXTON. Eye-Colour in Gammarus. (With Plate XIV and one diagram)	347

ERRATA.

THE INHERITANCE OF WING COLOUR IN LEPIDOPTERA.

I. *Abraaxas grossulariata* var. *lutea* (Cockerell).

On page 240, of Vol. VIII. No. 4, line 35, for '18 V read '18 Y

On page 241, line 21, for '18 V read '18 Y and

On page 241, line 29, for '18 V read '18 Y.

STUDIES IN THE HYBRID *BISTONINAE*.

IV. CONCERNING THE SEX AND RELATED PROBLEMS.

By J. W. HESLOP HARRISON, D.Sc.

(With Pl. I and 10 Text-figures.)

CONTENTS.

	PAGE
Introductory	1
On the wholly male broods	5
Inbreeding and its effects in inducing the appearance of females in broods which, according to expectation, should have been unisexual and male	13
The problem of the single female in the cross between <i>N. zonaria</i> female and a (<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀) male	16
On the preponderance of males in the <i>P. pomonaria</i> ♂ × <i>L. hirtaria</i> ♀ broods when the <i>L. hirtaria</i> were of Scotch origin	19
On the status of the <i>P. pomonaria</i> ♂ × <i>L. hybrid pilzii</i> ♀ brood	20
On the causes of the development of such intersexes	33
Description of the genitalia of one of the <i>P. pomonaria</i> × <i>P. lapponaria</i> intersexes	35

INTRODUCTORY.

EXPERIMENTS designed to secure hybrids in the Geometrid Subfamily *Bistoninae* have had sex results presenting unforeseen and abnormal occurrences of such diverse types that, whenever the general results of the experiments have been discussed, the sex problems have been reserved for special treatment; this treatment it is proposed to give in the present paper. Before proceeding with the discussion, however, as much of it depends on the phylogenetic relationship of the species concerned a brief sketch of the phylogeny must be given here.

As I have pointed out elsewhere the Geometrid Subfamily *Bistoninae*, on the structure of the genital armature combined with other more or less important characters, can be divided into two sections: (1) the

Non-Boarmioid Subgroup; (2) the Boarmioid Subgroup. At present we are only concerned with that small homogeneous portion of the former which includes the genera *Lycia*, *Poecilopsis* and *Nyssia*. Of these three, *Lycia* is fully winged in both sexes, whilst the other two are endowed with apterous females. From these circumstances alone it appears certain that *Lycia* is the oldest genus, although its primitive character may be confirmed in many independent ways. For instance, so closely is *Lycia* allied to *Megabiston plumosaria*, one of the generalised species at the head of the two subgroups, that if a female of that species were captured in England it would be regarded as a very minor aberration of *L. hirtaria*. Further, applying the well-known test of great discontinuity in geographical distribution to determine the age of a genus, we find that *Lycia* displays that phenomenon to a marked degree, its species existing in widely separated colonies in Europe, North Africa, Eastern Asia and Eastern North America—a range neither equalled in extent nor in discontinuity by either of the other genera *Poecilopsis* or *Nyssia*.

The genus *Poecilopsis* consists of four species, three being restricted to Europe and one to North America, whilst *Nyssia*, likewise comprising four species, is exclusively European. In outward appearance there seems to be little to suggest which of the two is nearer to the *Lycia* stem, but fortunately there are in reality several very important structural characters available as evidence, and nothing is more weighty than that supplied by the genitalia. Considering the male armature of all eight species we find that they fall into two groups, one with rounded valves comprising *Poecilopsis pomonaria* and *P. isabellae*, and the other with pointed valves including the remaining half dozen species.

Making due allowance for size, those of the former pair are indistinguishable from those of *L. hirtaria* whilst those of *P. lapponaria* and *P. rachelae* approach those of *Nyssia zonaria* in much the same way. This feature in itself forges a chain linking up two species of the genus *Poecilopsis* with *Lycia* and two with *Nyssia*, thereby assuring us that *Poecilopsis* has been derived from *Lycia*, and *Nyssia* from *Poecilopsis*.

On the other hand all four *Poecilopsis* species, in common with *Lycia*, have the male vesica armed with strong cornute, those on the same organ in *Nyssia* being obsolete or obsolescent; similarly in the female genitalia *Poecilopsis* agrees with *Lycia* in the possession of an arrowhead-shaped signum on the bursa copulatrix, and this signum *Nyssia* lacks entirely. These two observations combined emphasise the essential unity of the genera *Poecilopsis* and *Nyssia* within themselves,

as well as the attachment of the former to *Lycia*. Nevertheless, many additional points in which *P. rachelae* and *P. lapponaria* resemble *Nyssia zonaria*—like the larger ova, the indistinct larval pattern, the delicate texture of the larval skin, the shape of the wings of the imago—demonstrate that the *lapponaria-rachelae* pair although, as we gleaned above, veritable members of the genus *Poecilopsis*, are truly transitional between their congeners and *Nyssia*. So, too, the agreement in the ova, larval pattern and pupae between *L. hirtaria*, *P. pomonaria*, and *P. isabellae*, emphasised in no uncertain way by the fertility of the hybrids between them, proves that the last two are derivatives of *L. hirtaria*.

To sum up our facts, we are now in a position to state that the genus *Lycia* has yielded *Poecilopsis*, which in turn, *via* the species *P. rachelae* and *P. lapponaria*, has given rise to *Nyssia*, although the exact relationship of all the species within the three genera has not been stated. This need not detain us long. *L. ursaria* and *L. hirtaria* are what are termed "representative" species, *i.e.* are forms not widely apart, evolved from two sections of the same original, severed by some geographical accident or incident, *ursaria* from its pattern being nearer the early form. *P. rachelae* and *P. lapponaria* are likewise the isolated fragments of a Preglacial species thrust apart by the pressure of the oncoming ice as the Glacial period moved to its climax. On the contrary, that *P. pomonaria* and *P. isabellae* have diverged in more modern times, and that *P. pomonaria* is the older, are indicated by the complete fertility of their hybrids, by the superior fertility of the hybrids between *hirtaria* and *pomonaria* over the corresponding *hirtaria-isabella* hybrid, and by the attachment of *P. isabellae* to larch (*Larix decidua*)—a Post-glacial immigrant into Europe. *Nyssia alpina*, *N. graecaria* and *N. italica* bear a somewhat similar relationship to *N. zonaria*, having arisen in a common offshoot of that species subsequently split by isolation due to glacial action into the present assemblage.

Very briefly in this way a phylogenetic tree such as appears in No. III of the present series of papers has been worked out, and since it has been given there it need not be repeated.

For ease in reference, before the discussion is given, details of the sex results must be summarised, and they appear in the classified tables below.

*Crosses in which the two Sexes appeared in Proportions not requiring
Particular Discussion.*

Female	Male	Imagos	
		Males	Females
<i>Poecilopsis pomonaria</i> ...	<i>Lycia hirtaria</i> ...	98	92
<i>L. hirtaria</i> (English) ...	<i>P. pomonaria</i> ...	86	75
<i>P. isabellae</i> ...	<i>L. hirtaria</i> ...	38	32
<i>P. lapponaria</i> ...	<i>L. hirtaria</i> ...	20	19
<i>L. hirtaria</i> ...	<i>Nyssia zonaria</i> ...	94	200*
<i>P. isabellae</i> ...	<i>P. pomonaria</i> ...	23	24
<i>P. pomonaria</i> ...	<i>P. isabellae</i> ...	31	30
<i>P. pomonaria</i> ...	<i>N. zonaria</i> ...	44	102
<i>P. lapponaria</i> ...	<i>N. zonaria</i> ...	Excess of females	
<i>P. rachelae</i> ...	<i>N. zonaria</i> ...	15	13
<i>L. hirtaria</i> ...	<i>P. pomonaria</i> ♂ × <i>L. hirtaria</i> ♀ ...	6	4
<i>L. hirtaria</i> ...	<i>L. hirtaria</i> ♂ × <i>P. pomonaria</i> ♀ ...	10	7
<i>L. hirtaria</i> ...	{ (<i>L. hirtaria</i> ♂ × <i>P. pomonaria</i> ♀) ♂ × <i>L. hirtaria</i> ♀ }	115	103
<i>L. hirtaria</i> ...	{ (<i>P. pomonaria</i> ♂ × <i>L. hirtaria</i> ♀) ♂ × <i>L. hirtaria</i> ♀ }	123	113
<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀	<i>P. pomonaria</i> ♂ × <i>P. lapponaria</i> ♀	9	7
<i>P. isabellae</i> ♂ × <i>P. pomonaria</i> ♀	<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀ ...	11	9
<i>P. isabellae</i> ...	<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀ ...	3	2
<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀	<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀ ...	15	13
<i>P. isabellae</i> ♂ × <i>P. pomonaria</i> ♀	<i>P. isabellae</i> ♂ × <i>P. pomonaria</i> ♀ ...	14	16

Crosses Yielding Males only.

Female	Male	Male Imagos
<i>N. zonaria</i> ...	<i>L. hirtaria</i> ...	208
<i>N. graecaria</i> ...	<i>L. hirtaria</i> ...	65
<i>N. zonaria</i> ...	<i>P. pomonaria</i> ...	90
<i>N. zonaria</i> ...	<i>P. lapponaria</i> ...	93
<i>N. zonaria</i> ...	<i>P. isabellae</i> ...	32
<i>N. zonaria</i> ...	<i>P. pomonaria</i> ♂ × <i>P. lapponaria</i> ♀	All

Crosses Yielding Abnormally a small percentage of Females.

Female	Male	Males	Females
<i>N. zonaria</i> ...	<i>P. pomonaria</i> (inbred) ...	71	7
<i>N. zonaria</i> ...	<i>P. lapponaria</i> (inbred) ...	62	3
<i>N. zonaria</i> ...	<i>P. pomonaria</i> ♂ × <i>P. isabellae</i> ♀	17	1 (half-sized)

Cross giving an Abnormal Number of Males.

Female	Male	Males	Females
<i>L. hirtaria</i> (Scotch)	<i>P. pomonaria</i>	190	14

Crosses Yielding the Sexes almost equal in Numbers but with Odd Intersexes.

Female	Male	Males	Females	Intersexes
(1) <i>P. lapponaria</i>	<i>P. pomonaria</i>	38	39	1
(2) <i>P. lapponaria</i>	<i>P. pomonaria</i>	About equal		1

Cross Yielding Males and Intersexes only.

Female	Male
<i>N. zonaria</i>	<i>P. rachelae</i>

Cross giving only Intersexes.

Female	Male
(<i>P. pomonaria</i> ♀ × <i>L. hirtaria</i> ♂)	<i>P. pomonaria</i>

* In later experiments figures approaching equality have been obtained, the totals being 87 ♂♂, 79 ♀♀.

On the Wholly Male Broods.

In my experimental facts, tabulated above, an extraordinary and striking series of phenomena appears before our eyes. We see a suggestive chain of broods, commencing with those perfectly normal in their sex proportions, and passing through broods containing males and intersexes to those comprising males only. Furthermore, irrespective of any bizarre way in which a given brood may be built, that yielded in the reciprocal cross is, except that in two or three cases¹ the number of females is in excess, precisely what one expects in families of normal parentage.

Nor must it be supposed that these observations refer merely to certain selected families; it is not so. We are not here concerned with phenomena of family value, for whenever these crosses are made, provided that the same species and the same sexes are involved, the results are identically the same. And in some instances the number of such broods reared reaches a very high total, verging upon the outcome of over a hundred different pairings.

Even a cursory inspection of the sex numbers reveals the significant fact that, no matter what the constitution of the remainder of the brood is, in the case of the primary hybrids the males make their appearance in their accustomed numbers. They seem not to be affected in the slightest by the disturbing cause, whatever it may be, bringing into being these remarkable eccentricities.

The females, however, appear, in some anomalous and unexpected fashion, to have been urged progressively toward maleness, culminating, in extreme cases, in the tilting of the entire female organism into the male condition.

Concisely, the following statement enables us to gain a clearer idea of the stages whereby the final result is attained, and incidentally shows that, even before the actual sex is put in a state of unstable equilibrium, the interference with the full sex powers has already been initiated.

(1) *Crosses with both sexes partially fertile.*

Poecilopsis pomonaria female \times *Lycia hirtaria* male.

P. isabellae female \times *P. pomonaria* male.

P. pomonaria female \times *P. isabellae* male.

(2) *Sexes in the usual proportions: ova deposited but never hatching.*

Poecilopsis isabellae female \times *Lycia hirtaria* male.

¹ One very often rears a great excess of females in wild broods of the various species of the genus *Poecilopsis*, and occasionally in *Nyssia*.

- (3) *Sexes in the ordinary numbers, but no ova ever deposited.*

Poecilopsis lapponaria female \times *Lycia hirtaria* male.

- (4) *Cross reproducing the main features of the last section but, in addition, occasional intersexes appear.*

P. lapponaria female \times *Poecilopsis pomonaria* male.

- (5) *Crosses yielding males and intersexes.*

P. rachelae female \times *Lycia hirtaria* male.

Nyssia zonaria female \times *Poecilopsis rachelae* male.

- (6) *Crosses which, when under normal conditions, yield males only.*

Nyssia zonaria female \times *Lycia hirtaria* male.

N. graecaria female \times *L. hirtaria* male.

N. zonaria female \times *Poecilopsis pomonaria* male.

N. zonaria female \times *P. isabellae* male.

N. zonaria female \times *P. lapponaria* male.

The circumstances outlined in the first two paragraphs are quite consonant with one's expectations in the study of hybrids; nor are those recorded in the third paragraph a matter for surprise, for they are a natural corollary to those preceding them. But one was quite unprepared for the occurrences mentioned in the last three.

In searching for a plausible interpretation of these facts, so utterly unexpected, and so absolutely, on the face of the matter, at variance with one's preconceived notions obtained from the Mendelian theory of sex, let us see if we can correlate any further facts with observed phenomena.

Careful examination of the species makes it plain that whenever the broods are abnormal the female is almost invariably provided by *Nyssia zonaria*; if it is not, and this occurs in two cases only, then the female is that of *P. lapponaria*, the species from which *N. zonaria* is evolved, or of *N. graecaria*, a form derived from it. On the other hand, the male is uniformly that of a phylogenetically older insect than the female. Furthermore, referring only to the anomalous families, the nearer the genetic connection between the species concerned, the fewer the number of aberrant intersexes making their appearance.

Obviously, in the reciprocal crosses, in which no sex-weakening is developed, the cross is, of necessity, between a phylogenetically older female and a younger male.

As has been demonstrated in Doncaster's work on sex-limited inheritance in *Abraxas grossulariata* and in my own on *Oporabia*

autumnata, in the Lepidopterous family Geometridae the males, as regards sex, are homozygous and the females heterozygous. Consequently the males of the whole of the species must be represented as of constitution XX ; similarly that of the female is XY ¹. Therefore the gametes of the male are all of type X , and of the female X and Y , appearing in equal numbers. Naturally then, on fertilisation, whether the gametes taking part proceed from the same or diverse species, two types of zygotes are generated, one type being of composition XX and the other of formula XY . In pure species the former are the males, and the latter manifest themselves as the females; and the same holds true, when no sex disturbance occurs in the products, of the hybrids. When such interference does appear, the males still remain of the type XX whilst the affected zygote, no longer to be recognised as a female, nevertheless bears the sex chromosome combination peculiar to that sex. It follows immediately from this that, when the sex genes in any given zygote are not those of one and only one species, a mere setting up of the XY system does not in itself suffice to ensure that that zygote should develop into an organism of female type. Or, in terms of the chromosome theory (and almost certainly the actual explanation) of heredity and sex, whether we regard the so-called sex chromosomes as synonymous in whole or in part with the sex genes, the possession of an X chromosome inherited from a phylogenetically older male, and a Y from a younger female, does not indicate that the individual carrying them is a female. An immediate deduction from this is that the male sex gene or chromosome of the older form is able, not only to neutralise, but to overpower, either completely or partially, that from a female of later development. Whenever this overpowering takes place wholly, we have a complete conversion of zygotes of build XY' into males. Such an occurrence happens in the *hirtaria* \times *zonaria* and accompanying crosses. In other words, it seems that the sex chromosome designated " X " of the older insect has been able to supply enough sex chromatin, or is powerful enough in itself (the two statements are not synonymous) to set up for the hybrid a system ending in the complete passage of a female into a male. Or, symbolically, considering a zygote of form XY' produced in the hybridisation of phylogenetically old males with young females, the X is potent enough to destroy the effects of the Y present and also to set up in its place a modified (and therefore better written **XX**) XX system

¹ X is used to designate the male gene or factor and Y the female, whilst X' and Y' are used for similar genes in the case of a second species.

which, for the hybrid, suffices to cause it to appear as a male. And this necessitates the acceptance of the corollary that the male sex gene or determiner in *hirtaria* exceeds in power the corresponding factor or chromosome in *zonaria*.

Now this prepotency may, conceivably, be brought about in two ways; either by the gross physical factor of mere size acting as a twenty gram brass weight weighs down and thus overpowers a similar ten gram weight, or it may rather be a matter of intensity of action working somewhat as a small platinum weight turns the beam against a brass weight of double its size.

If the former be the case, then a cytological examination of the chromosomes of *hirtaria* and of *zonaria* should produce evidence corroborative of the point. Make the necessary examination, and what do we find? We discover that the haploid chromosome number of *hirtaria* is 14 and that of *zonaria* 56, the individual chromosomes of *hirtaria* being *nearly* always larger than those of *zonaria*. But note the significant figures of 14 and 56. The ratio between these numbers is 1:4 and it would imply that the chromosomes of *hirtaria* are complexes, each carrying genes equivalent to those borne by four of *zonaria*.

From this one must conclude that only part of one *hirtaria* chromosome provides the mechanism of the sex determination. But it is not impossible that one of the larger *zonaria* chromosomes and one of the smaller ones of *hirtaria* are those responsible for the sex determination, and in size these are so nearly alike as to remove any impression that size alone will account for the preponderating influence of the *hirtaria* male factor. Nor is this incompatible with the significance of the figures 14 and 56, for it seems quite possible that the complexes of *hirtaria* are not invariably groups of four units—if we term a *zonaria* chromosome a unit. There are more methods of making four than by adding together two and two!

In my opinion, the balance of the evidence indicates that we must look to the intensity of the sex factors for an explanation of the aberrant results.

Now this may be merely a case of the stronger force of a *hirtaria* X chromosome overcoming that of the weaker Y from *zonaria*; on the contrary, it may be that sex is a matter of potential, and that in the X and Y chromosomes of any given being we are concerned with something of a nature comparable with positive and negative electricity. In an ordinary pure species we can regard the X chromosome as

carrying a force parallel with positive electrical potential, and the *Y* as bearing a similar analogy to negative. From this view of sex, the male is essentially a creature of high sex potential as carrying two like factors, whereas a female, with two equal unlike factors, is one of neutral or zero sex level. To illustrate (not that the figures have any real value), suppose we assign to an *X* chromosome of *hirtaria* a sex potential of +50, and to a *Y* chromosome of the same origin the value -50. Then, on fertilisation, the sex level of a zygote destined to be males would be +100, whereas that of the females would be 0.

But there are many grades or stages between 0 and 100, from which it appears possible that all males are not necessarily exactly alike; nor are the females uniformly the same. There must be some scope, even if not great, for oscillation around the values 0 and 100. Our next task will be to determine what would happen if by suitable manipulation we obtained a series of organisms lying around the 50 level and shading off in both directions, one lot toward zero and the other to 100; clearly the former are tending to be females and the latter males. This suggests that the forms close to the 50 value are to be regarded as neither males nor females; in other words, they must be intersexes.

Granting that such is the case, and that such potential differences are at the root of sex determination, and granting also, as an allowable deduction from the experimental evidence in the *hirtaria-zonaria* crosses, that the values to be assigned to the sex genes vary with the species, then it ought to be perfectly feasible, by some happy hybrid combination of species, to produce insects endowed with a sex potential which, as far as the hybrid is concerned, stands at the mean in question. Careful study of the experimental data yielded by the crosses named above makes it practically certain that, as we pass away phylogenetically from *hirtaria*, the powers or potential of the male sex factors diminish, and that of the female varies correspondingly. Hence, crosses between two Bistonine forms, utilising males of phylogenetically older insects and females of later origin, may give us confirmation of these views. We chose, as a start, forms such as *pomonaria* male (the older form) and *isabellae* female, and crossed them. In the actual experiments, the result was that the sexes emerged in equal numbers. Certainly from that we cannot extract any facts likely to throw light on the problem, and the reason is plain; *isabellae* is a form of very recent development from *pomonaria*, and their sex values have not diverged to any appreciable extent. Laying aside this experiment as not being of importance in so far as concerns our present quest, we took

up the next available pair. Again choosing the older male, and selecting a female of more recent production, or rather evolution, I mated a *pomonaria* male with a *lapponaria* female. As a result of the first trial I obtained the following sex proportions, 38 males and 39 females and one intersex, and, what is more striking, in a further experiment made to supplement this one, once more another intersex made its appearance accompanied with equality in the numbers of genuine males and females bred.

Apparently these broods place us in possession of confirmatory facts of great value in pursuing our investigation. The sex potentials in the two species have not been so far apart as to destroy to any great extent the normal equality of the two sexes in numbers. Yet interference has occurred, and that it is not accidental we discern from the appearance of a single half or intersex in each of two distinct broods.

Now these intersexes give us information of value, not by their advent alone, but by their actual sexual condition, as is made clear by the following chain of reasoning. Since, in producing males according to the theory propounded above, we are always fusing gametes of like high sex potential, zygotes likewise of high potential should result and such can scarcely be anything else but males. On the contrary, in the fusion of gametes derived from different species and of unlike potential (the outcome of which in ordinary pure forms is the production of females of approximately zero sex level), the result is not of necessity low; it may actually, as we see by employing figures such as +50 and -20, be comparatively high.

Zygotes of this value, ordinarily females, may make an approach to maleness and some, attaining the mean between the two alternatives, may be thrown to some degree towards it. If approach of the type indicated here was caused in the present broods, according to the scheme laid down, then the insect displaying it should, if we attach due weight to the almost normal sex proportions, be predominately female; that such is really the case may be seen by a glance at Text-fig. 10.

Proof of this kind only makes it the more certain that, conceding the validity of our arguments, if we can make a combination affecting the sex to this extent, further possible combinations should result in the tilting of, not one or two, but of the whole of the females into the condition of intersexes. For testing this, many more species remain and were utilised. A male *rachelae* (older) was paired with the

phylogenetically younger female *zonaria* and the results justified the most optimistic expectations, for the brood was entirely composed of males and intersexes. Once more only one explanation rises to one's mind, viz. that, as a result of combining gametes both of high "positive" potential, we have generated a cumulative potential in the zygote high enough to secure maleness. On the other hand, the fusion of gametes, one of high male possibilities and the other of lower female powers, has induced a sex potential in the zygote of such a level as to raise it away from the female level, high enough to render it an intersex but not sufficient for maleness. Again, if theory be consistent with fact, an examination of the sexual character of the intersexes should betray, by the overwhelming nature of the female leanings, that these intersexes were modified females, and such indeed was the case.

Still a crucial test is left; if it be possible to incline all the females so far from femaleness as "intersexuality," then it must be possible to throw them wholly into the male condition. To make the trial we have still older species to employ, and these were paired in the usual combination of the older male with the female of later evolution. *Hirtaria* males were mated with *zonaria* and the still younger *graecaria* females; the broods were successfully reared, and they comprised males only. Now half of these males must be normal ones of sex chromosome content XX' , but the other portion, males in every structural essential, can only be in possession of chromosomes necessitating the sex formula of the individuals in it being written XY' . In connection with this it might be urged that selective viability had been at work suppressing the female zygotes; this cannot be so, for in carefully conducted experiments all of the ova proved fertile, and were successfully reared to the imaginal state. Another answer to such objections, if one were required, is the presence of the intersexes in the families considered previously.

Therefore, almost certainly, the experimental results make it imperative that sex should be deemed a matter of potency or potential, the latter the preferable as supplying a better explanation of the development of intersexes. Further evidence pointing in the same direction may be deduced from the sex proportions of the reciprocal crosses in every case; in these no anomalies in sex ever appear. Males and females alike make their appearance in their accustomed numbers just as if the broods were those of pure species making their appearance naturally. Let us consider how the potential theory works out here. We pair *zonaria* male (younger) with *hirtaria* female (older). For

simplicity and conciseness in the study, let us assign arbitrary values to the potential of the sex genes (or chromosomes) of the two insects. Suppose we assume the powers of the male sex gene of *hirtaria* to be of value 50 and, consequently, that of the female as - 50. Then, since we have discovered by experiment that those of *zonaria* must be fixed at a lower figure, let their values be 20 and - 20 respectively. On these assumptions the XX' zygotes produced carry the high potential of 70 and are therefore males, while the XY' carries the abnormally low one of - 30; this, whatever it may be, certainly makes no approach to maleness and consequently is a female; and its extraordinary low sex level may be the cause of its experimentally proved sterility. Again there is harmony between fact and theory and, what is still more weighty, this harmony extends over all known crosses of a similar type.

Although my inclinations lie towards the acceptance of the scheme developed above as being the most satisfactory explanation of the sex phenomena, it is not the only one springing to my mind.

In fertilising ova of one species with the sperm of another, it is necessary to note that the mere mechanical passage of the spermatozoa through the ova is not a matter of normal conditions of environment. In particular, on account of the great disparity in size between those of *hirtaria* on the one hand and those of *zonaria* on the other, *hirtaria* sperms have a much longer distance to travel than in ordinary acts of fertilisation. This means greatly delayed fertilisation; that such delay did result was specially obvious in the *hirtaria-graecaria* eggs, for so great a time intervened between the passing of the sperm through the micropyle and the fusion of the nuclei that the ova collapsed precisely as in the case of a non-fertilised egg. Postponement of this nature implies over-ripe eggs; and over-ripeness in the eggs of other groups has ended in wholly male cultures. Similar causes, induced in varying ways, may produce similar effects, and over-ripeness may explain the aberrant sex results.

There is also another set of circumstances possible through the presence of strange spermatozoa in the ova. It may happen that the effect of this is to cause the sex chromosomes to behave uniformly so that the Y chromosome (or even both X and Y) is constantly forced to the opposite pole to the intruding sperm, and thus to be invariably extruded with the last polar body. In the former event, the egg nucleus would always contain a male determiner or chromosome, whilst the latter would yield ova void of sex determination; in which case, fer-

tilisation would generate zygotes of composition XX or XO , the former assuredly males and the latter, if viable, also males. Nor is there anything to prevent their being viable, for zygotes, proved cytologically to be provided with an X chromosome alone, have been encountered in Bridges' *Drosophila* cultures, and these were abnormal only in their sterility.

The difficulty, however, in both of these explanations seems to lie, and more especially under the supposition that over-ripeness explains the anomaly, in the fact that at first sight they offer nothing to account for the intersexes. Nevertheless, over-ripeness may lead to diminished vigour and vitality of the ova and a concomitant loss in power of the sex genes which, in organisms with heterozygous females, would more particularly affect that sex toward indifference and possibly intersexuality.

Under the second supposition there is also a feasible possibility, and that is that the force urging the sex chromosomes to be included in the polar body may be inadequate to force it far enough away in the anaphase; in which case it may be that a mechanical entanglement occurs, trapping the Y chromosome between the egg nucleus and that of the polar body, ending in the inclusion of part of it in the ovum and part in the polar body—to me a very unlikely event. If such did happen, the egg would be deficient in sex chromatin of the Y nature. Consequently, the zygote proceeding from it would be unable to set up in its entirety the system required to induce femaleness and a half- or intersex appears.

To conclude, whilst the last two theories offer some explanation, my preferences lie, as stated, toward the potency theory, especially that phase of it which represents sex as a matter of varying potential.

If this be the case, then an easy extension enables one to explain the curious sex results in Shull's *Lychnis dioica* families and in Correns' hybrids between *Bryonia alba* and *Bryonia dioica*, whilst a parallel theory has been evolved already independently by Goldschmidt to account for the results in Brake's mongrels between *Lymantria dispar* and its Japanese local race var. *japonica*.

Inbreeding and its Effects in inducing the Appearance of Females in Broods which, according to expectation, should have been Unisexual and Male.

Owing to the great difficulties entailed in obtaining frequent supplies of either *Poecilopsis pomonaria* or *P. lapponaria*, I have

resorted to very close inbreeding of these two species extending over a period of several years. Such inbred material, on account of the risks involved, was never used so long as sturdy wild insects were procurable, and this was not always possible. Occasionally therefore, in cases of emergency, I was driven to the use of my own stock, and the first case in which such a procedure was adopted was in a pairing between *P. pomonaria* male and *Nyssia zonaria* female, a cross-pairing which, in the usual course of events, should have produced nothing but males; nevertheless, in the resulting brood, a few females put in an appearance. At the time not much importance was attached to the occurrence, it being assumed to have arisen through an accidental admixture of larvae with those of the reciprocal cross during some changing operation. In the succeeding season resource had to be made to a similar stock of inbred *P. lapponaria* for securing the parallel cross. Again a few females appeared abnormally; this could be no longer imputed to accident. Two definite series of parallel experiments were consequently initiated; in one set the male parents were closely inbred and, in the other, wild stock was employed, but, in all instances, *N. zonaria* provided the female. Once more the experimental issue was that, when wild males were used, the families obtained were uniformly unisexual inasmuch as they contained males only; on the other hand, with the other material, a few females aberrantly emerged. Not only were such cultures made involving inbred *P. pomonaria* and *P. lapponaria* but, in addition, further hybrid broods were reared with strongly inbred *Lycia hirtaria* as the male parent: here, however, the outcome was the usual unisexual male broods; nor has any subsequent similar attempt, no matter of what duration the inbreeding of the *hirtaria*, affected the sexual composition of the families obtained by cross-pairing *L. hirtaria* male and *N. zonaria* female.

Diametrically opposed to this, without exception, whenever I have utilised the males of the inbred *hirtaria* allies, the result has remained the same; females, restricted in number, have been secured. Possibly, though such attempts were not practicable during the war, still closer inbreeding of *hirtaria* may ultimately determine the breeding of females in the *hirtaria* male and *zonaria* female cross. At present my limited supply of *N. zonaria* is ear-marked for the *rachelae-zonaria* trials which are of greater importance.

Only one conclusion can be deduced from these consistent experimental observations, and that is that inbreeding has a very profound effect in weakening the male sex determiners in *P. pomonaria* and *P. lapponaria*.

Let us digress here for a moment to consider the course of events when inbreeding is persisted in within the limits of one species. During the period between the years 1906-1912, for purposes quite remote from the circumstances under discussion, I kept going a race of *Zonosoma orbicularia* which, on account of the great rarity of the species, could not be otherwise than inbred, and inbred extremely closely. At first, despite the fact that I was rearing two and sometimes three broods per annum, the race seemed to retain its wonted vigour; but, in 1910, abnormal sex ratios were manifested coupled with a decided diminution of both vigour and of sexual instinct, cases of reluctance to pair, even with the conditions at the optimum, being quite usual.

And the unequal sex ratios were in the direction of an excess of females. Still, inbreeding was resorted to until, in 1911, several of the broods were wholly female in composition, while yet certain of the remainder contained a few males. Although these, in the main, refused to pair, they supplied enough potent males to ensure the continuance of their race until the ensuing year, when it ended in the production of females only, involving, of course, the extinction of the stock.

Now let us deliberate as to what this may mean. Only one inference can be extracted from it, that the power of the male sex factors has, by degrees, been diminished by inbreeding until at the end it has become in its effects wholly negligible. In other words the sex potential of the male gene (or rather of the chromosome bearing it) falls, brood by brood, until it becomes practically zero and thus the final zygotes, as regards sex level, are in the neutral position reflected in those zygotes by what we call femaleness.

What is the bearing of this on the results with which we are now concerned?

A theory has been formulated above that the aberrant unisexual broods are consequent upon the disparity in sex potential of the male and female sex genes, or of the chromosomes in which they are located; in particular, it was deemed proved that the male potential of the phylogenetically older *P. pomonaria* and *P. lapponaria* was great enough to overwhelm the power of the female sex genes, or *Y* chromosome, of the younger *N. zonaria*. But if, as a result of the inbreeding of the former pair of species, the potential of the male element falls, it is obvious that if its fall progresses with the degree to which we carry the inbreeding, it may sink low enough to fail to overcome the effect of the female determiner of *N. zonaria*. Consequently, in certain zygotes, there will occur no setting up of the pseudo-*XX* condition expressed

in the zygotes by their maleness; therefore females will appear as in ordinary broods, although it appears feasible enough, and is to be expected in the long run that, with repeated experiments, these females may, should the exact sex potential arise, be replaced by intersexes in odd cases. And these theoretical deductions are exactly in accord with the facts; the use of inbred *pomonaria* and *lapponaria* did yield a small percentage of females.

We shall now proceed to discuss the persistent failure, up to the present, to upset the unisexual state of affairs in broods obtained by hybridising *L. hirtaria* male and *N. zonaria* female. It was laid down that, with the phylogenetical divergence from *hirtaria*, an accompanying decrease of the potential of the male sex determiners was encountered. Clearly then to begin with, *pomonaria*, and still more emphatically *lapponaria*, should be inferior to *hirtaria* in such powers. Whence, on inbreeding, both should fall much sooner to that level which fails to destroy the power of that sex chromosome lodged in the (under ordinary conditions) female-producing ova of *N. zonaria*. Thus females should appear much sooner in these cases than with *hirtaria*, and, inferentially, much more readily with *lapponaria* than with *pomonaria*. Of the truth of the latter statement, experimental proof was readily secured. From this argument it seems certain that if the inbreeding of *hirtaria* be pursued far enough, ultimately the *hirtaria* male and *zonaria* female cross will result in the production of a limited number of females.

The problem of the Single Female in the Cross between N. zonaria Female and a (P. pomonaria ♂ × P. isabellae ♀) Male.

The advent of the single female in this brood may suggest that it is susceptible of explanation on the same, or similar, grounds to those advanced to account for the few females reared in the *P. pomonaria* ♂ × *N. zonaria* ♀ and *P. lapponaria* ♂ × *N. zonaria* ♀ hybrids; this cannot be so. The reduction of the potencies of the sex genes by inbreeding plays no part in the matter, the male used being of hybrid origin. In parentage this male was of wild blood on both sides, the *pomonaria* male coming from Munich in Bavaria, and the *isabellae* female having been bred from wild larvae taken at Innsbruck in the Tyrol. This diverse nature of the parents was reflected in the offspring, for the hybrid male was of enormous size, its wing-expanse being 33 mm. as against 32 mm. for *pomonaria* and 31.5 mm. for *isabellae*.

Hybridity or heterozygosis, as opposed to the enervating effects of inbreeding, has a building up or stimulating influence on the hybrid organism physiologically. Should progressive sterility not be displayed, and it was not in the special case we are discussing, this heterozygosis of the *pomonaria-isabellae* hybrid should so affect the sex genes as rather to increase their values in comparison with the stationary factors of *zonaria* than diminish them.

This increased vigour, due to its heterozygous nature, had other expression in the insect than in size; it was betrayed in the greater manifestation of the sexual instincts which caused all the hybrids of this origin to pair instantaneously with any female with which they were enclosed. The copulation with the *zonaria* female lasted for the prolonged period of 36 hours.

The chromosome complement of the two species *P. pomonaria* and *P. isabellae* is almost alike, the former species being endowed with a haploid number of 51 and the latter of 52. In the gametogenesis of their hybrids so homologous are the chromosomes that almost uniformly a perfect reduction division takes place. Even had it not done so, that fact, by its very occurrence, would give us an extremely forceful argument against any interference with the anticipated sex yield of unisexual male broods only. In the event of no reduction, the spermatozoa of the hybrid male, in lieu of being supplied with one male sex gene or chromosome, would carry two, and as a necessary consequence would be doubly endowed with factors active in upsetting the power of the female sex determiner in the female-producing ova of *N. zonaria*.

Again, this unlooked-for female was quite abnormal in size; it barely attained one half that of the pure species. To make this the more emphatic the insect was weighed, as were also males and females of the pure form and, to ensure accuracy, the weighing was undertaken while the insects were as yet in the pupal state. The following were the respective weights:

Weight of abnormal hybrid female	14 grm.
Weight of hybrid males	23 grm.
Mean weight of the females of <i>P. pomonaria</i> , <i>P.</i> <i>isabellae</i> , and <i>N. zonaria</i>	27 grm.

From this table it is apparent that the female in question is practically one half of the weight one would have anticipated. This argues or rather indicates that something has occurred to prevent the development of the insect to its full size. What caused this phenomenon was,

in all probability, the active agent in procuring the sex reversal. In other words, we have to seek for some event which, at one and the same time, dislocates the sex system and likewise brings into being circumstances ending in a half-sized imago.

Suppose the female-producing ovum which gave rise to this insect to have been fertilised by a spermatozoon containing, as a result of an ordinary reduction division, one X chromosome or gene. In composition the zygote would be represented as XY , for we must not lose sight of the fact that, in spite of the zygote's being a male, in these hybrids it carries the sex chromosomes typical of a female when found in pure species. Imagine that, in the first cleavage, there is a mitotic dislocation leading to the passage of an undivided X chromosome to one pole and of a like unsplit Y chromosome to the other, this happening owing to their failing to take part in the usual division. Then the cell on one side endowed with the X chromosome would, if viable, tend to induce the appearance of male characters on that side whilst that on the other would, again if viable, by virtue of its unique Y chromosome, end in the appearance of female structures. Thus, if both survive, the outcome would of necessity be a lateral gynandromorph, probably both genetically and somatically.

If, on the contrary, a cell of the type XO cannot exist, then we have left a cell of composition YO , obviously of female possibilities, to continue the perfection of the organism. That such a destruction of one of the first cleavage cells does not imply a suspension of development but rather ends in the appearance of beings of inferior size has been proved often enough by the artificial removal of such a cell.

This differential viability of cells of the types XO and YO has been definitely proved by Bridges' cytological investigations as to the chromosomes of certain flies in his *Drosophila* cultures, known to be of untypical chromosome content. He found, however, that it was the XO zygotes that could continue to develop whereas the YO 's were incapable of existence. We must note, however, that his experiments involved *Diptera* in which the females are sex homozygotes and the males, on the other hand, heterozygotes—exactly the reverse to what obtains in the *Bistoninae*. This assures us that, in our case, the preferential survival may be in favour of the cell YO . Incidentally, it indicates that the function of the Y chromosome is not that of a mere foil to the X chromosome in the mechanism of the reduction division, but that, as demanded by the potential theory of sex, it has a real positive and functional value in the matter of sex determination.

There is yet one possible method of mitotic dislocation of the sex chromosomes. It must not be lost sight of that we are here discussing a hybrid insect and, therefore, that the conditions in mitosis are not normal. Suppose that the *Y* chromosome, owing to the composite build of the cells, is late in dividing, and, as a result, the split halves lag long enough on the spindle to be enmeshed in the protoplasm of one daughter cell alone; on the contrary, imagine the *X* chromosome to divide normally. Then we should obtain two cells, one in character *XO* and the second *XY'Y'* (using *Y'* to designate the split halves of the original *Y* chromosome). Then, if once more the viability of the cells were not alike, and the *XO* cell were incapable of existing, we would have the work of perfecting the organism thrown upon one of the first cleavage cells alone and that the one of sex chromosome value *XY'Y'*. In this, through the inability of the split *X* to overcome the effects of the two halves of the *Y*, the cumulative potencies of the latter pair might result, if segmentation proceeded, in the building up of a female.

Even assuming that, exceptionally, in the gametogenesis of the *pomonaria-isabellae* hybrids the sex chromosomes refuse to pair in the reduction division, and thus the fertilisation of a *zonaria* female-producing ovum yields a zygote including three sex chromosomes and is therefore of composition *XXY*, mitotic dislocation of the same type can still produce a female. It is quite conceivable that, in the *pomonaria-isabellae-zonaria* zygote, owing to the presence of a multiplicity of chromosomes in a cell intended for the movements of 56, they impede one another in mitosis and produce a disjoining of the sex chromosomes ending in the continued existence, as before, of cells of female type alone.

A study of the above arguments will show that the progressive degradation (or uplift) of the females through intersexes into males in the primary hybrids referred to above may not inconceivably be due to some incompatibility between certain chromosomes and the cells containing them; though this, with due regard to the perfect transition chain, is not very likely.

On the Preponderance of Males in the Poecilopsis pomonaria Male × Lycia hirtaria Female Broods when the L. hirtaria were of Scotch Origin.

A further puzzling sex phenomenon was the huge excess of males in the *hirtaria* ♀ × *pomonaria* ♂ broods when the *hirtaria* used were of Scotch origin, in contrast with the equality of the sex numbers when the *hirtaria* were English.

This simply means that accompanying the racial difference there must be a physiological divergence, and this was confirmed most strongly by the significant difference in the products of the back cross between hybrid *pilzii* male and *hirtaria* female when the *hirtaria* blood in the *pilzii* was of Scotch origin, and when it was from another source.

With this physiological difference, or rather as a special item in it, the observed facts state unequivocally that a distinct alteration of the powers of the sex genes has occurred, so that, in crossing *pomonaria* males with Scotch *hirtaria* females, we are not reproducing the circumstances attending similar crosses in which English *hirtaria* are concerned; we seem rather to be imitating the conditions of the crosses between *hirtaria* ♂ and *zonaria* ♀ which, as we saw, yielded wholly male families.

If this explanation of anomalous broods be valid, i.e. that it depends on a racial variation of the powers of the sex determiners, then it is reasonable to suppose that there must exist other local races of insects displaying similar discrepancies in sex potencies, and such is the case as Brake, Poppelbaum, Goldschmidt, and Muschamp found in their mongrels between *Lymantria dispar* and its Japanese local race var. *japonica*. What seems of special import in this instance was the obtaining of intersexes.

On the Status of the P. pomonaria Male × L. hybrid pilzii Female Brood.

Before proceeding to the consideration of the intersexes obtained as the progeny in this cross, it will be well to glance for a moment at the structure of the genitalia of both sexes in this group. Fortunately, this is simple in both cases and need not detain us long.

Male structures. Text-fig. 1, male genitalia of *L. ursaria*.

In this figure, diagrammatic almost in its simplicity, will be noticed a terminal pointed portion; this is termed the Uncus and it represents the tergite of the 10th abdominal segment. Attached to its base appears a chitinous collar provided with a chin-like projection impressed with scale-like hollows; to this organ the name Gnath or Gnathos is applied. Just anterior to the uncus is the Cingulum or Tegumen Ring replacing the 9th segment; the basal or sternal portion of this structure is thrown forward into the abdomen to form a kind of hollow or pouch known as the Saccus. Articulated more or less with this tegumen are the Penis and its members. The penis, i.e. the whole organ, Aedoeagus, vesica, cornuti combined, may be seen lying to the left of the figure, between the uncus and the left valve or clasp. Projecting from the

aedoeagus may be perceived the Vesica, armed near the orifice with a comb-like band of spines which we call the Cornuti.

To the right and left of the genitalia are spread the two Valves (Valvae) or clasps; these also are strongly chitinised. Toward what, in the sketch, seems to be their inner margin, they are raised and thickened; to this inner margin the name Costa is given.

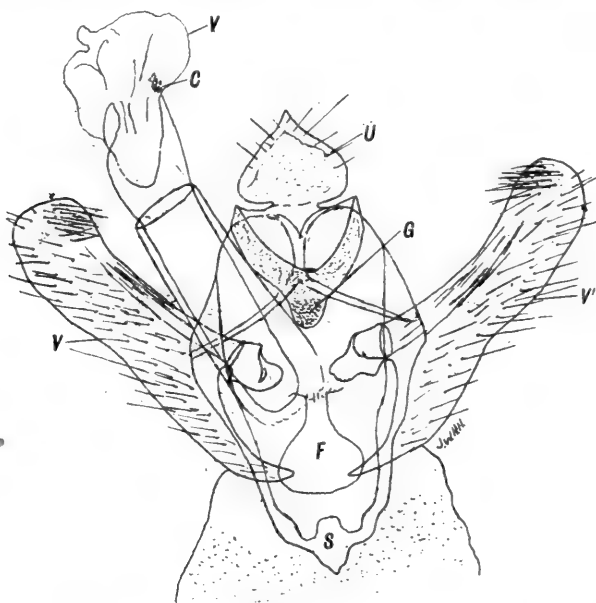


Fig. 1. Male genitalia of *L. ursaria*.

U = the Uncus. V, V' = the Valves. G = the Gnathos. F = the Furca.
S = the Saccus at the base of the Tegumen. V = the Vesica. C = the Cornuti.

On the whole, the stiff armature of bristles spreads outward or rather, when the structures are *in situ*, downward. A small patch more closely set, however, at the tip points in the reverse direction. This minute patch characterises the whole sub-family and is of considerable phylogenetical importance.

Basally, between the two valves can be made out a thin flask-shaped plate of chitin, probably, when functional, of use as a penis guide.

Female structures. Text-fig. 2, female genitalia of *L. hyb. hulli*.

For similar simplicity I have chosen to figure the abdomen of a female hybrid *L. hyb. hulli*.

On account of the enormous development of the ovipositor, the segmentation of the female abdomen, which, in the majority of Lepi-

doptera, is obscure and, therefore, exceedingly difficult to make out, is here much simplified.

At the end of the abdomen may be seen the bristle-tipped ovipositor lobes to which are attached a pair of stout black rods called the inner directing rods, the function of which is to secure the insertion of the ovipositor in nooks and crannies suitable for the reception of the ova. This portion represents the female 10th abdominal segment. Anterior to this part is another section likewise armed with a pair of directing rods; this is all the female has in the way of a 9th segment.

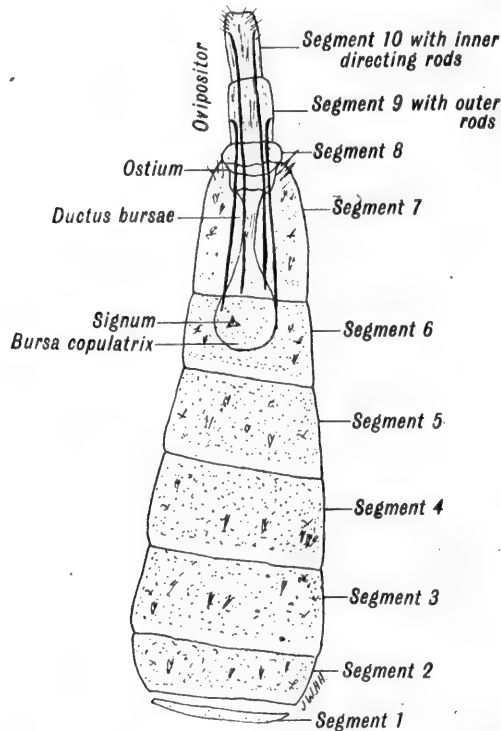


Fig. 2. Female genitalia of *L. hyb. hulli*.

In most Lepidopterous females the 8th segment appears to be obsolete. In this sub-family it presents a more or less distinct tubular or collar-like structure bearing anteriorly the orifice of the female genital pouch. To this opening the name Ostium bursae, or simply Ostium, is applied; in the *Bistoninae*, it is armed with a thin chitinous plate or guard flap. Leading to the Bursa copulatrix proper from the ostium is a narrow tube, the Ductus bursae, from which issues the Ductus seminalis. The

Bursa copulatrix itself is a small bag or pouch, sub-globular in shape. At one part of its inner surface it becomes heavily chitinised and a structure formed like a flint arrow-head is evolved. To this I gave the name "Sagitta"; simultaneously, Pierce termed it the "Signum." As Pierce's name has priority in publication it will have to be adopted.

Genital structures of the intersexes.

(1) Wing parts, Pl. I, Fig. 1. Genitalia, Text-fig. 3.

As might have been anticipated from an inspection of its external features this specimen displays in its genitalia a much greater development of male organs than do the others. Practically every male structure superimposed on, or fused with, those of the female is to be recognised.

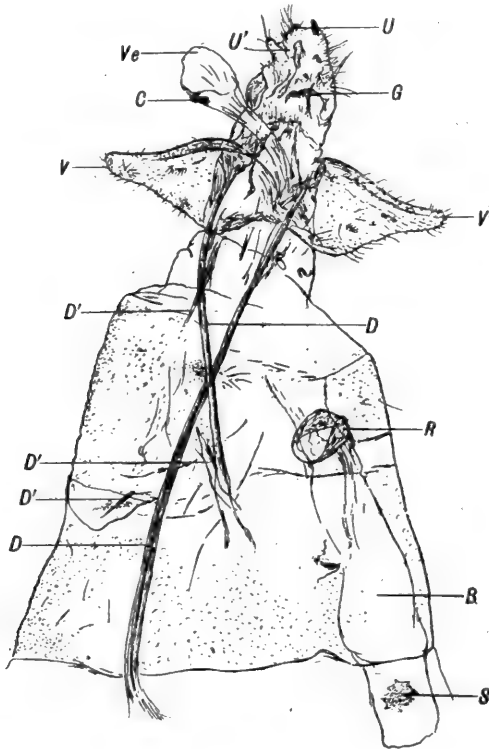


Fig. 3.

B=Bursa copulatrix. C=Cornuti. DD=1st pair of directing rods.
D'D'=2nd pair of directing rods. G=Gnathos. R=Duplicated
8th abdominal segment. S=Signum. V, V'=Valves. Ve=Vesica.
U=Uncus. U'=another portion of uncus.

Terminally appear the two ovipositor lobes bearing, as is invariably the case in all of the intersexes I have examined, a small detached portion of the uncus (*U*), a further fraction of which occurs at *U'*. Immediately to the left of this is an attempted duplication of the ovipositor. Fused with the latter organ at *G* is the squamous gnathos tip. Curiously enough, this curious stranding of the tip of the gnathos is the normal state of affairs in certain *Bistoninae*, e.g. *Haggardia subalbata* (Warren). Close to the gnathos are two minute chitinous rods or bars; these are the rudiments of the tergite of the 9th abdominal segment of the male or, otherwise, they represent the "roof" of the tegumen.

Connected with the base of the ovipositor posteriorly are visible the inner pair of directing rods almost normally placed. However, they are so situated as to suggest their homology with the ventral section of the male tegumen ring. Similarly, the uniform occurrence of the uncus at the ovipositor tip indicates that these structures are homologous, and that both are developments of the 10th abdominal segment.

Half-way down the ovipositor are to be found the two male valves, fully developed, if abnormal in shape. These possess the ordinary thickened and raised costa and likewise the inturned patch of stiff bristles on their outer extremity. Thrown to the left between them appears the aedoeagus, on the vesica of which are the cornuti of a fully sexed male.

To the left of the first pair of rods are the degenerate remains of the second pair, the left member of which is divided into two parts, one small lying at the base of the 7th segment and a further more important portion traversing the left upper rod above. Of the right lower rod a mere fragment crosses the same rod at its base.

Just to the left of the larger detached portion of the second pair is the ostium with its guard-flap well displayed; there, too, may be seen a part of the female 8th abdominal segment. Passing from it is the ductus bursae in a very elongated form, ending in a bursa copulatrix much less globular than usual but, nevertheless, exhibiting a well-developed, somewhat circular, granulated signum, armed at the edges with a series of small spines or spiculations.

Precisely in front of the spiracles of the 6th segment, and resting on the ductus bursae, is a strong free chitinous ring; *this is a duplicated 8th abdominal segment*. That occurring at the correct point is the corresponding segment of the female; the present is that of the male. Though small, it is still perfect in shape. Its internment is necessitated by the essentially external nature of the ostium bursae.

(2) Wing parts, Pl. I, Fig. 2. Genitalia, Text-fig. 4.

Here we have an insect of almost identical type with the preceding but, nevertheless, exhibiting a much less decided attempt at a combination of the two types of sexual organs. Those of the male are suppressed to a greater extent and, in consequence, an accentuation of the female structures is to be seen.

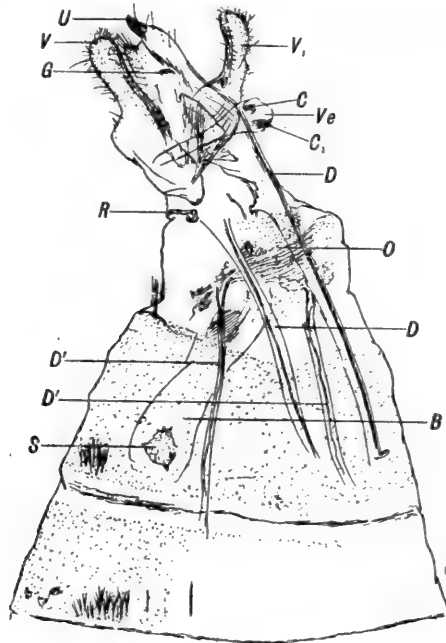


Fig. 4.

B = Bursa copulatrix. *CC*₁ = Duplicated cornuti. *DD* = 1st pair of rods.
D'D' = 2nd pair of rods. *G* = Gnathos. *O* = Ostium. *R* = Duplicated 8th abdominal segment.
S = Signum. *V, V*₁ = Valves.
Ve = Vesica. *U* = Uncus.

As before, at the extremity of the abdomen lies the ovipositor armed at the tip with a fragment of the male uncus. In the present case, however, instead of being central, the uncus is dextral; thus, in its development, it has not interfered with that of the left ovipositor lobe nor has the latter affected it. Naturally then, both have reached a more perfected state than in the specimen already described. The left tip is therefore quite female and the right similarly male.

Immediately in front of the uncus is a small portion of the gnathos, and to the right of this again may be made out a very small area of the 9th abdominal tergite.

Proceeding from the uncus base and merging insensibly into the first pair of directing rods is a thin chitinous structure evidently the upper lateral portion of the tegumen. Once more we gain proof of the homology of the saccus, and the ventral parts of the tegumen, with the directing rods. Next, as in the first insect, are the two valves distorted, it is true, to a slight extent but approximating more closely to the normal form. Between them, and lying to the right, is the aedoeagus, the vesica of which is ornamented as usual with cornuti but with this anomaly that they are here duplicated.

The lower pair of rods call for no particular comment; nor is there much reason for specially noting the 8th female abdominal segment. Again, however, this segment is doubled by the advent of a second segment possessing the characteristics of the same segment of the male. The extra segment is not so perfect as in the last instance and appears as an irregular chitinous ring at *R*. Nor is the ostium bursae so plain but it is to be found on the right just above the lower rods. In this insect, the ductus bursae is shorter and the bursa copulatrix itself more globular. On its inner surface is the somewhat larger signum, the spines of which are thicker and stronger than in the last specimen.

(3) Wing parts, Pl. I, Fig. 4. Genitalia, Text-fig. 5.

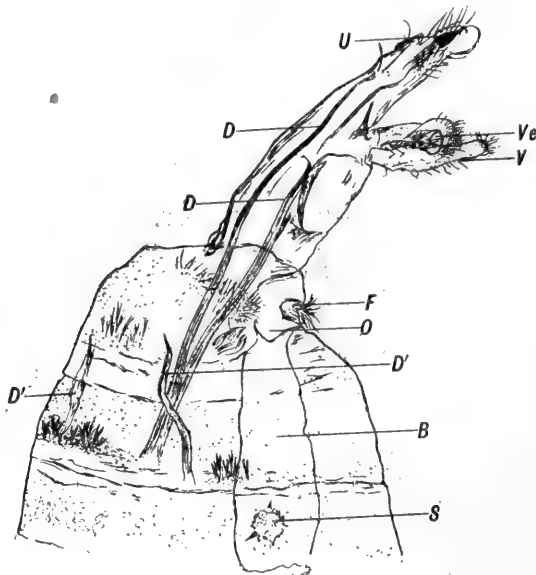


Fig. 5.

B = Bursa copulatrix. *DD* = 1st pair of rods. *D'D'* = 2nd pair of rods.
F = Fragment of duplicated 8th segment. *O* = Ostium. *S* = Signum.
V = Valve. *Ve* = Vesica. *U* = Uncus.

A further loss of the male element is here encountered. The ovipositor tip is very nearly normal but it still carries in the median line a small, but rather perfect, uncus.

Of the two upper directing rods, the left is as usual, but the right is interrupted by the sudden appearance of male valves attached as a sort of appendage to the penultimate ovipositor segment. Of these two valves, the right is reduced in size although otherwise structurally perfect; the left, on the contrary, seems larger and distorted.

Between them appears the penis, the vesica of which, in this instance, bears no cornuti.

Immediately beneath these male valves is the continuation of the fractured rod.

The ostium bursae, ductus bursae, and signum now approach the normal, and the duplication of the 8th segment is almost, but not quite, avoided. Whilst the 8th female segment is practically uninterfered with, some trace of the male influence can still be seen in the extraordinary retention of the two tubercles which characterise the external male *pupal* genitalia.

Although slightly weakened, the lower pair of rods are much the same as in a pure bred insect.

(4) Wing parts, Pl. I, Fig. 3. Genitalia, Text-fig. 6.

Once more the ovipositor lobes are normal and yet we have grafted on them, as it were, detached portions of the uncus, one of which resembles that described in studying the creatures already considered. The other is a very small isolated fragment situated on the left. Whilst the usual pair of 10th segmental rods are almost as usual, matters are complicated by the development of a single male valve to the right of the ovipositor. In normal Lepidopterous males the valves are hinged or attached on either side near the articulation of the tegumen. There being no tegumen here, unless a somewhat thickened area in the ovipositor ranks as such, we have nothing to which to articulate the anomalous valve. This difficulty seems to have been avoided in the building up of the hybrid insect by the advent of an extra directing rod which forms the base of the valve, and then passes anteriorly as a fifth rod.

At the base of the ovipositor appears a sac, in which can be seen chitinous plates armed with huge masses of long stout hairs; these are the male valves which, for some reason, fail to reach the external surface and lie free in the abdominal cavity.

Let us consider for a moment the cause of this. These valves lie free in the abdomen and are quite shut off from any connection with the exterior; yet, to judge from their armature of hairs, one would look upon them as ectodermal in character.

In development, they arise from a body first dissected out by Herold years ago. This little body or corpuscle, whilst apparently simple in structure, is duplex in origin; one part arises as an invagination of the

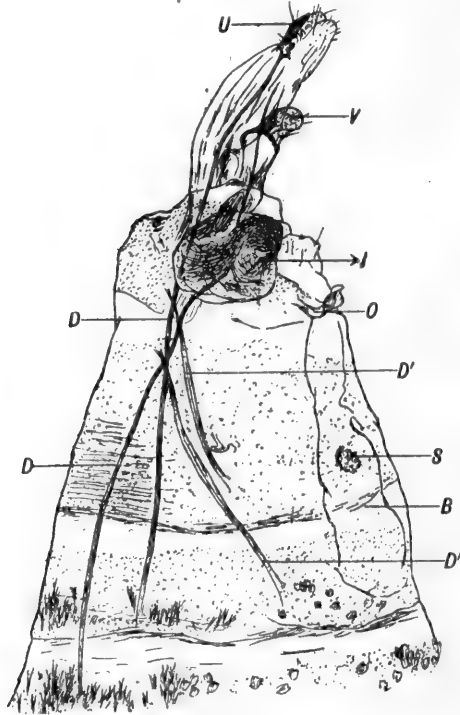


Fig. 6.

B=Bursa copulatrix. DD=1st pair of rods. D'D'=2nd pair of rods.
 I=Valve enclosed in pupal covering. O=Ostium. S=Signum.
 U=Uncus. V=Valve.

posterior margin of the 9th abdominal segment and is therefore dermal in derivation. On the contrary, the remaining portion is not of such origin as it develops from a group of cells at the base of the larval genital ducts.

From the invaginated cells, ectodermal in character, are evolved the two valves which are consequently dermal structures as might have been determined otherwise from a consideration of their vestiture of hairs and bristles. The other section of the corpuscle, not of dermal origin,

gives birth to structures such as the penis and its attendant organs which, necessarily, show their internal character by displaying no signs of hair or bristle development.

Clearly, since the clasps once more migrate to the surface when the insect emerges from the pupa; nay, since they betray their presence externally in a suppressed manner in the familiar tubercles of the pupal genitalia, they must have retained therewith some connection of a guiding nature directing them to the surface when such a passage is imminent.

Such a connection we see hinted at, in these intersexes, by noting, whenever the valves appear externally, their invariable, significant, and intimate attachment to the penultimate section of the female ovipositor which we have indicated as representing the 9th abdominal segment.

But why in our insects should the valves fail to pass to their normal position? Simply because their position externally is taken up by that part of the ovipositor homologous with the male 9th abdominal segment.

As these creatures are fundamentally females, such structures as are essentially female find preferential expression in the pupa and finally in the imago. If the clash between the two sets of organs is slight and they develop *pari passu*, then the second ovipositor segment and the valves alike appear; if the male element however is weakened in its expression, or is retarded otherwise in its appearance, then the female structures, utilising this start, bar the way to the surface and, of necessity, the male organs remain inside.

This is equivalent to asserting that, if in a normal male we can induce artificially the development of a mass of tissue to act as an obstruction to the outward passage of the valves, then they ought to remain inside as in my specimens. Such interference may be obtained by making a small cicatrix at the critical point between the 9th and 10th abdominal segments as in Chapman's experiments with *Lymantria dispar*, the results of which were (minus the female structures) precisely the same as those described in these hybrids.

To return to the general characters of the third insect but little remains to be said. On the left side the ostium bursae, the ductus bursae, bursa copulatrix and signum are alike complete, and as in an average Bistonine female.

(5) Wing parts, Pl. I, Fig. 3. Genitalia, Text-fig. 7.

In this instance, the entire mass of the genitalia is unusually crowded together. Exactly as in the preceding insects, the uncus

retains its place between the two ovipositor lobes; in spite of this, the latter are quite complete as are also their pair of directing rods.

Massed in the seventh segment, and lying free therein, are the valves and, as in the last case, these still remain almost, although not quite, in the pupal condition. One valve seems to have made its way to the exterior and is to be seen superimposed on and lying parallel to the anterior part of the ovipositor.

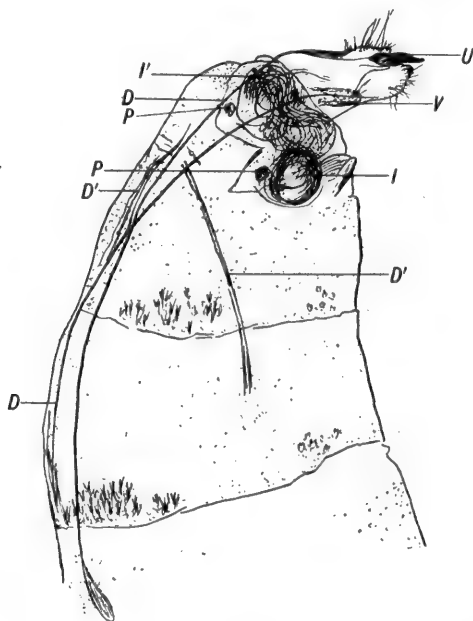


Fig. 7.

DD=1st pair of rods. *D'D'*=2nd pair of rods. *II'*=Duplicated and enclosed valves. *PP*=Pupal tubercles. *U*=Uncus, *V*=Valve.

The two pupal tubercles marking the points at which the valves should have gained access to the outside may be detected at *I*.

The ostium lies to the left but the other female parts, unless overwhelmed in the valves and their enormous hirsute covering, defy detection.

(6) Wing parts, Pl. I, Fig. 5. Genitalia, Text-fig. 8.

In this case, as far as these sexual structures are concerned, an almost perfect approach to femaleness is made.

We have a perfect ovipositor correctly furnished with a normal pair of outer directing rods, the only unusual feature being the appearance

of a small portion of the uncus at the tip and a small part close beside it.

Segment nine, although retracted, is similarly perfect as are also its rods. Just within the left rod is the ostium bursae with its guard-flap, and leading away from it in a complete state are the ductus bursae and the bursa itself which is decorated internally with a signum of ordinary type.

Placed one on each side of the left inner rod are to be noted the pupal tubercles.

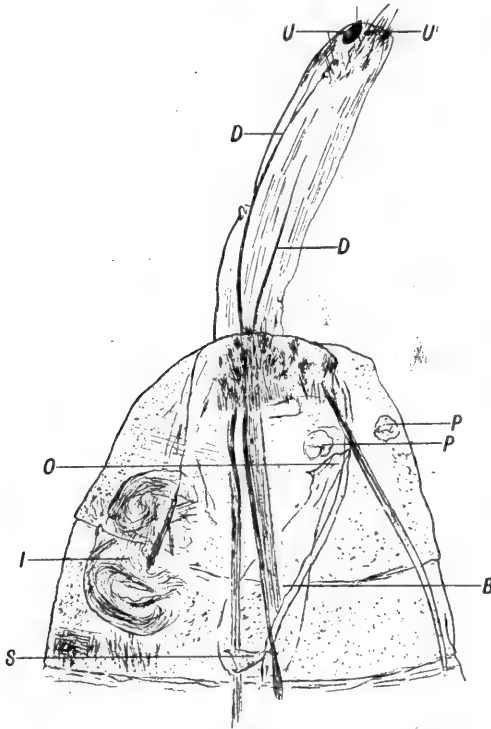


Fig. 8.

B = Bursa copulatrix. *DD* = 1st pair of directing rods. *I* = Enclosed
valves. *O* = Ostium. *PP* = Pupal tubercles. *S* = Signum.
U = Uncus. *U'* = Detached fragment of uncus.

On the right, half in the 7th segment and half in the 6th, are the valves again wrapped in the pupal envelope and, on account of the perfection of the female organs, unable to secure exit.

In spite of their density and entangled character they can be made out to be two distinct structures.

(7) Wing parts, Pl. I, Fig. 6. Genitalia, Text-fig. 9.

This is another example that seems almost wholly female.

The ovipositor, seemingly perfect, possesses on both right and left lobes a distinct pointed uncus fragment. Both of the outer directing rods are placed as usual and so too is the right inner one. The left inner rod, however, is defective and appears to the left as black twisted chitinous structure.

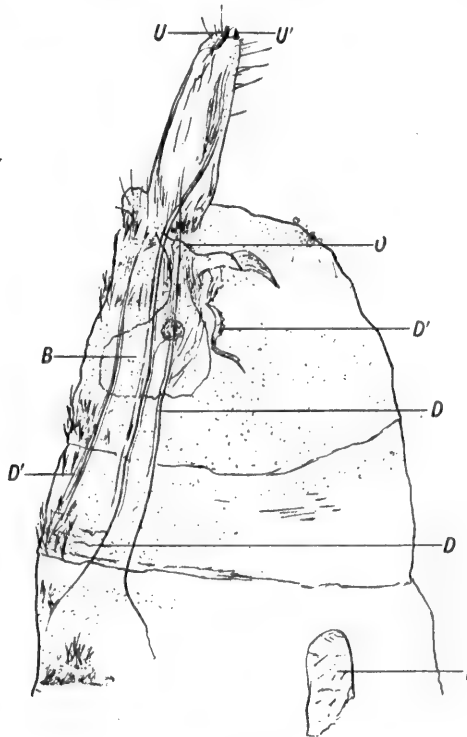


Fig. 9.

B = Bursa copulatrix. *DD* = 1st pair of rods. *D'D'* = 2nd pair of rods.
I = Enclosed valve. *O* = Ostium. *UU'* = Fragments of uncus.

Of the male valves but one can be detected, and that lacks the basal portion. It lies loose in the body cavity and somewhat anteriorly. Except that it is fragmentary, it is certainly not misshapen.

The ostium appears to the left of the main rods and a bursa of sorts proceeds from it. Whether the rounded dark patch, apparently within it, is the signum, is a moot point. To me it is the pupal tubercle pertaining to the valve which is inside. Had it been the signum, with

due attention to the predominantly female nature of the insect, one would have anticipated that it would have been arrow-head shaped.

On the Causes of the Development of such Intersexes.

Before we consider the *raison d'être* of the present series of intersexes the course of events in the oogenesis of the pure species of this subfamily must be examined.

At a very early stage indeed in the oocytes destined to result in ova, the homologous chromosomes pair; amongst those so doing are the sex chromosomes. If the female, as in our group, is heterozygous for sex, then the *X* chromosome behaves as the homologue of the *Y* and pairs with it in synapsis. All of the chromosomes remain paired until long after the yolk has been laid down and the egg extruded from the oviduct—nay, even after the spermatozoon has entered it. A division spindle is finally formed and the chromosomes separate, one half set proceeding to one pole and the other half set to the other pole of the spindle. From this, it must be obvious that only one of these daughter nuclei contains the *X* chromosome and one the *Y* when the female is a sex heterozygote.

Immediately these reach the poles, they form two new spindles in which, instead of having paired chromosomes at the equatorial plate, we have single chromosomes which then split as in the ordinary division of a somatic cell. The outermost pair of little new cells, composed of but little else save chromatin, are extruded as the first polar body. A similar fate awaits the outermost group of chromosomes formed as a result of the division of the original inner series of whole chromosomes; it forms the second polar body. It will now be perfectly clear that the innermost nucleus of all, which is the real egg nucleus and that which fuses with the spermatozoon, is furnished with an *X* or a *Y* chromosome, but not with both. Therefore, on fertilisation by the sperm derived from a male sex homozygote, it can only yield in the normal course of events one fixed sex.

But whether any given sex chromosome passes in the first maturation division to the outer or inner pole of the spindle is purely a matter of chance; whence it happens that, since two chromosomes enter into the matter, the chances that any particular egg is endowed with an *X* or *Y* are equal. Consequently, the zygotes formed consist of approximately equal numbers of those of male and of female type.

Turning now to the oogenesis of hybrid females, such as our *pilzii*, what differences are perceptible?

In the maturation divisions of such insects the progress of events is greatly altered. Owing to the fact that chromosomes derived from parents of diverse species are not always homologous, many chromosomes fail to find mates in the first divisions. Then at the point when in normal oogenesis the paired chromosomes separate, to ensure cell division, the unpaired chromosomes in hybrid oogenesis split. Thus, both in the egg and in the first polar nuclei, we find varying numbers of chromosomes which, in extreme cases like ours, attain the full somatic number. Both egg and polar body, in the case of a hybrid female heterozygous for sex, carry a complement of two sex chromosomes.

Thus, as in the spermatogenesis of hybrid *pilzii* males, in the oogenesis of hybrid *pilzii* females no genuine reduction division occurs.

It also appears that, as regards sex chromosomes, the ova of hybrid females agree in being the possessors of two, and thus incapable of yielding on fertilisation zygotes of two types as are the ova of sex heterozygotes of pure species.

When these abnormal ova are fertilised, as in the case of *pilzii* by *pomonaria*, by spermatozoa carrying uniformly an X chromosome, on fusion we get zygotes all alike and of composition $XX'Y$ (using X' to designate the *hirtaria* chromosome). Two of these, X and Y , are inherited from the same species and, being more prone to act in harmony, are likely therefore to be instrumental in settling the sex scheme. The possession of such a pair of sex chromosomes necessarily ends in femaleness. Therefore, the insect will be essentially female but thrown out of equilibrium by the presence of the extra X' . Now the cell containing this combination was built for, and contains the mechanism for dealing with, a normal number of chromosomes but, in place of holding a maximum of, say 100, it is supplied with nearer 116 some of which, those of *hirtaria* origin, are excessively large.

As a consequence, in the early cleavage divisions and probably also in many instances later, we have abundant scope for a failure of the usual chromosome splitting. Such mitotic dislocation we know to occur but not always in the same form. At one time it may be an X , and at another it may be a Y , that lags and passes undivided to one pole, and so on with every possible combination of divided and undivided chromosomes. As a result of mitotic accidents or incidents of this kind, we shall have produced at varying stages of segmentation cells of varying chromatin content as far as sex (and also other) chromosomes are concerned. If the Y goes undivided to one pole, the two daughter cells are of two types, one XX' and the other $XX'Y$, or, if an X and

the Y interfere with one another, then the daughter cells are of composition XX' and XY . In both cases the result is the same; we have adjacent to one another groups of cells, one of the male type XX' and the other of forms, XY and $XX'Y$, of female possibilities.

Side by side, therefore, structures typical of the opposite sexes are built up, and an intersex, or what has been termed a mosaic gynandromorph, will result. Clearly, however, there must be a majority of cases of cell division in which, as far as the sex chromosomes are involved, no dislocation occurs. Therefore, since, as was stated before, the insects are fundamentally females, they should remain so. And that such was the experimental outcome our examination of the sexual organs demonstrated.

Granting that this is so, since disjunction can occur at any point in segmentation, we have scope for every transition form between creatures almost wholly female and those displaying a mosaic of equal portions of both sexes, the latter as a result of mitotic dislocation in some early cleavage stage, and the former generated by later occurrences of the same nature.

It has been advanced by Standfuss and others that these intersexes in the *Saturniidae* and other families, are produced by the mere presence of three sex determiners in one zygote. Without dislocation this is impossible, for then the insects, whatever they might be sexually, would be uniform in structure and this we have seen is not the case. Such suggestions would not have been brought forward had dissections been made, and the actual condition of the intersexes been determined.

Besides, the direct cytological evidence of Bridges, who was fortunate enough to come into possession of a stock of *Drosophila* rejoicing in three sex chromosomes, showed that in such cases, exactly as I have postulated, there was a preferential and uniform setting up of one sex only. Only when, as in Morgan's experiments, dislocation occurred did mosaics result.

*Description of the Genitalia of one of the P. pomonaria × P. lapponaria
Intersexes. Text-fig. 10.*

In this insect, as must naturally occur from its origin, the femaleness is predominant. We see a fully developed ovipositor the right lobe of which is perfect in every respect whilst the left is replaced by an uncus

reaching a great pitch of development much greater than in the *pomonaria-pilzii* crosses. Both of the right directing rods are as fully formed as in a normal female but those of the left side are extremely feeble. The left outer rod, at its point of attachment to the terminal ovipositor segment, is thread-like but dense; as it passes anteriorly, however, it becomes feebler, thinner and broader. Of the latter type too is the left lower rod.

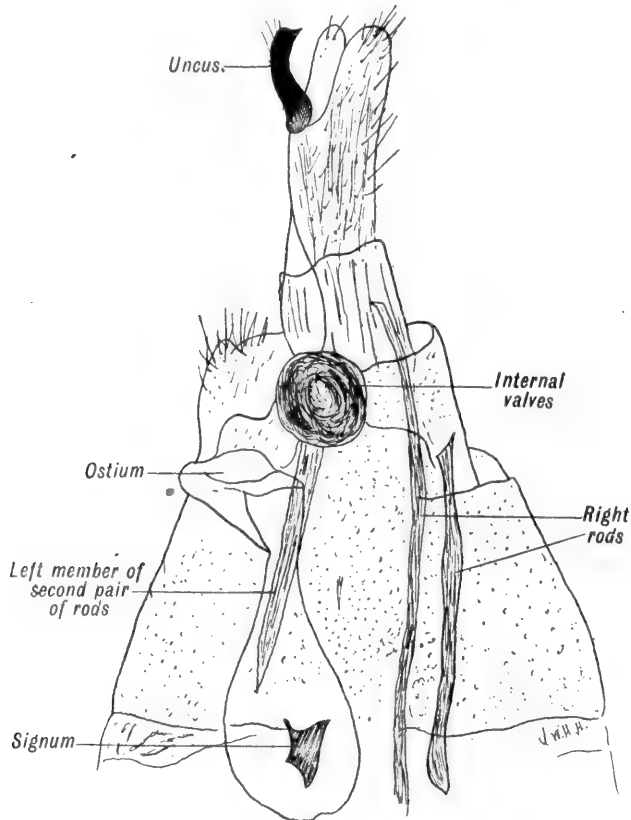
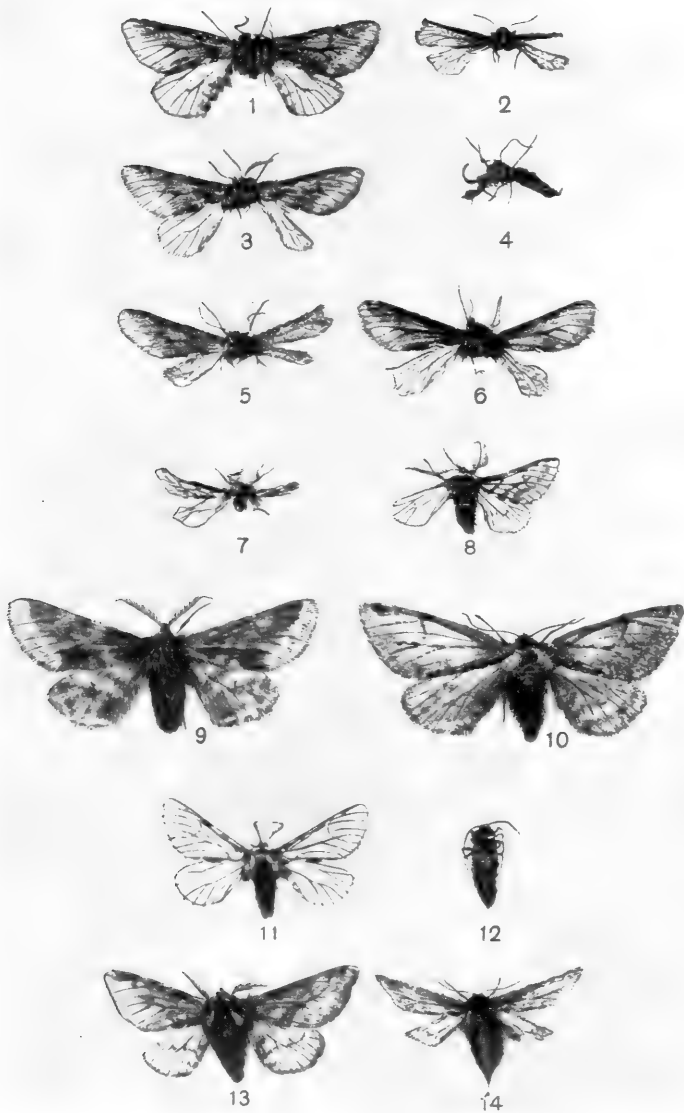
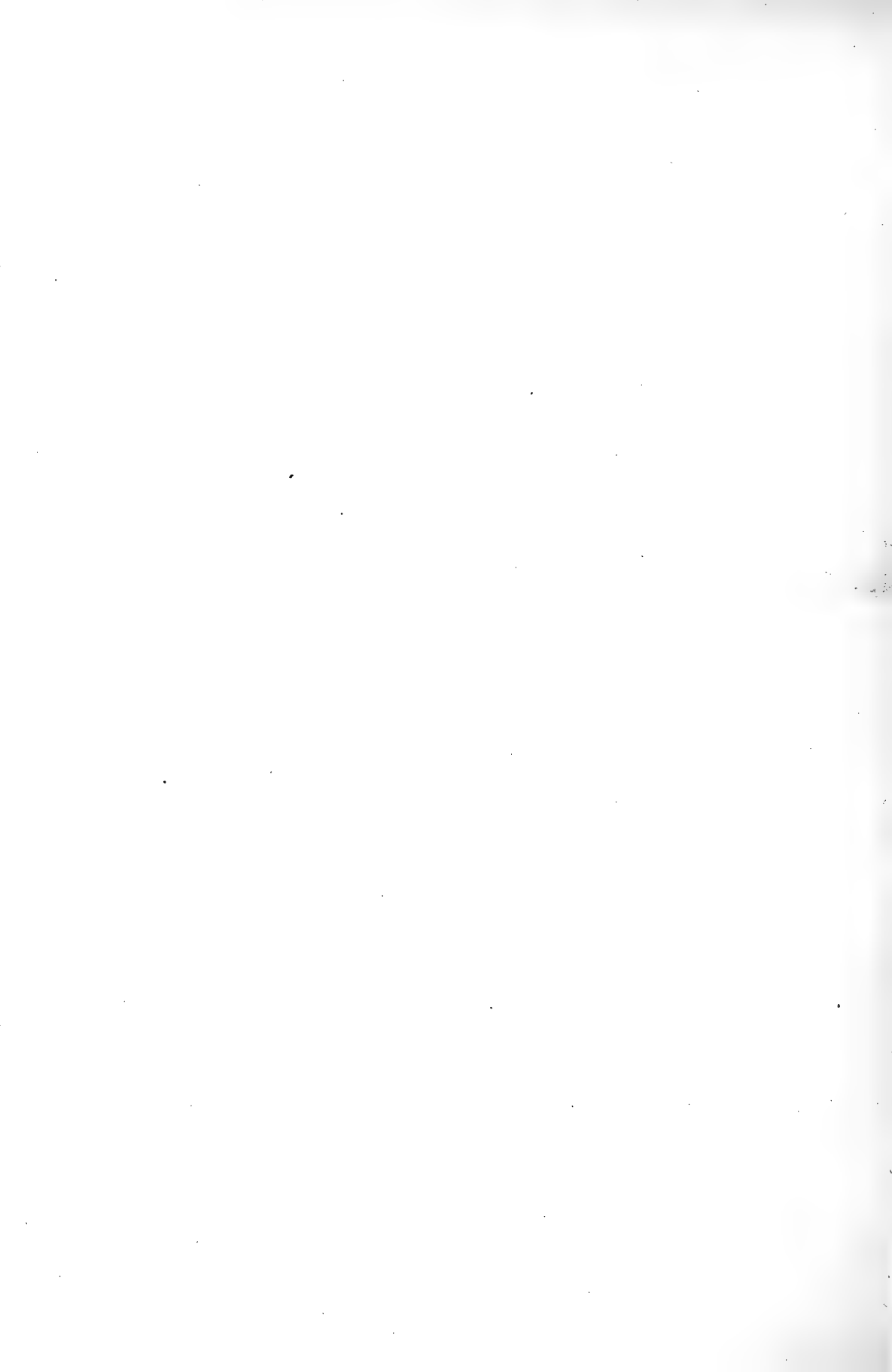


Fig. 10.

The ostium bursae is as usual but the ductus bursae and the bursa copulatrix itself are slightly shorter and somewhat distorted. The signum, however, is of the ordinary arrowhead-shaped type.

Placed centrally and a little above the ostium is a sac containing a miniature set of undeveloped male valves with bristles and hairs complete. Evidently they have been unable either to attain their full





size or to reach the exterior owing to the overpowering femaleness of the insect.

[I have to acknowledge the receipt of a grant from the Government Grant Committee of the Royal Society in aid of the above investigation.]

EXPLANATION OF PLATE I.

- 1, 2, 3, 4, 5, 6. Intersexes of pilzii (=pomonaria ♀ × hirtaria ♂) ♀ × pomonaria ♂.
- 7, 8. Intersexes of lapponaria ♀ × pomonaria ♂.
9. Male hirtaria. 10. Female hirtaria. 11. Male pomonaria.
12. Female pomonaria. 13. Male pilzii. 14. Female pilzii.

LITERATURE QUOTED.

- BRAKE. "Resultate der Kreuzung zwischen *Lymantria japonica* Motsch. und *Lymantria dispar*." *Entom. Zeitschrift*, 1907.
- BRAKE and FRINGS. "Einige weitere Ergebnisse der Brakeschen *Lymantria dispar* L. Zuchten." *Entom. Zeitschrift*, 1911.
- CHAPMAN. "An Experiment on the Development of the Male Appendages in Lepidoptera." *Transactions of the Entomological Society of London*, October 4, 1912.
- BRIDGES. "Non-disjunction as a Proof of the Chromosome Theory of Heredity." *Genetics*, Vol. I. pp. 1—52, 107—163. Princeton, January—March, 1916.
- CORRENS. "Die Rolle der männlichen Keimzellen bei der Geschlechtsbestimmung der gynodiozischen Blütenpflanzen." *Ber. deutsch. Bot. Ges.* 26a, 1908.
- DONCASTER. "On the relation between chromosomes, sex-limited transmission, and sex determination in *Abraxas grossulariata*." *Journ. of Genetics*, IV. No. 1, 1914.
- "The Determination of Sex." Cambridge Univ. Press, 1914.
- GOLDSCHMIDT. "Erblichkeitstudien an Schmetterlinge, I." *Zeitschrift für induct. Abstammungs- und Vererbungslehre*, 7, 1912.
- and POPPELBAUM. *Idem*. II. *Ibid.* 11, 1914.
- HARRISON. "The Hybrid *Bistoninae*." *Etudes de Lepidopterologie comparée*. Fasc. VII. Rennes, 1913.
- "Studies in the Hybrid *Bistoninae*." *Journ. of Genetics*, Vol. VI. No. 2, 1916.
- "The Geographical Distribution of the Moths of the Geometrid Subfamily *Bistoninae*." *Naturalist*, 1916, 1917.
- "Studies in the Hybrid *Bistoninae*, II." *Journ. of Genetics*, Vol. VI. No. 4, July 1917.
- "Genetical Studies in the Moths of the Geometrid Genus *Oporabia*." *Journ. of Genetics*, Vol. IX. (In the Press.)
- HERTWIG. "Über den derzeitigen Stand des Sexualitäts-problems." *Biolog. Zentralbl.* p. 1, 1912.

MORGAN. *Heredity and Sex*. Columbia University Press, 1914.

— "Mosaics, etc. in *Drosophila*." *Proceedings of the Society for Experimental Biology and Medicine*, Vol. XI. No. 7, August 1914.

— "Constitution of the Hereditary Material." *Proceedings of the American Philosophical Society*, Vol. LIV. No. 217, May—July, 1915.

MUSCHAMP. "Gynandromorphism in a Mongrel Brood of *Lymantria dispar* and its Race, var. *japonica*." *Ent. Rec.* Vol. XXVIII. No. 5, 1916.

SHULL. "Reversible Sex-mutants in *Lychnis dioica*." *Botanical Gazette*, Vol. LII. No. 5, Nov. 1911.

— "Inheritance of Sex in *Lychnis*." *Botanical Gazette*, Vol. XLIX. 1910.

STANDFUSS. "Mitteilungen zur Vererbungsfrage unter Herabziehung der Ergebnisse von Zuchtexperimenten mit *Agria tau* L." *Mitteilungen der Schweiz. entom. Gesellschaft*, Bd. XII. Heft 5/6.

A PRELIMINARY STUDY OF THE EFFECTS OF ADMINISTERING ETHYL ALCOHOL TO THE LEPIDOPTEROUS INSECT *SELENIA BILU- NARIA*, WITH PARTICULAR REFERENCE TO THE OFFSPRING.

BY J. W. HESLOP HARRISON, D.Sc.

I. INTRODUCTORY.

MY attention was first drawn to the present subject during the course of an examination of the facts published by Morgan and his pupils concerning their work on the fruit fly, *Drosophila ampelophila*. No one can study that work without being struck by the immense number of hereditary variations which have appeared, and continue to appear, in their cultures. So great and important are these when compared with the few similar mutations encountered in Nature that one is almost forced to the conclusion that in some way or other the technique employed is correlated with the observed results. Having conceived this notion, I submitted their methods to careful scrutiny but could only find one definite point in which their procedure differed from that followed by me when rearing lepidoptera or phytophagous hymenoptera or coleoptera, and that was that every individual fly used in the cultures had, at some period of its life, undergone etherisation; that fact naturally attracted suspicion. Moreover, these suspicions were strengthened when brought into conjunction with the wonderful tales one hears of the degeneracy in the offspring of alcoholic parents, as well as with the actual impairment of the young seen by Stockard in his alcoholised guinea-pigs. It thus seemed exceedingly probable that these effects were directly induced by the action of the ether on the germ cells of the parents at some very susceptible stage of their existence. Further, after careful consideration, it appeared in my view to follow as a natural corollary that if the single etherisation of the *Drosophila* flies were replaced by the continuous exposure of the organism throughout the

critical period of gametogenesis, from the earliest spermatogonial or oogonial division to the development of the perfect spermatozoon or ovum, results more important still might be obtainable. For such an experiment a lepidopterous insect, particularly a double brooded Geometer, was eminently suitable, and to carry it out a stock of the bivoltine race of *Selenia bilunaria* was secured.

Immediately after I had made these arrangements, Pearl's papers, in which he gives complete details as to his experiments on the administration of methyl and ethyl alcohols and ether to poultry, came to hand. So different were the results described there from those of Stockard, and so apparently contradictory to them were they, that it almost seemed impossible for one to harmonise them in the slightest. Stockard found that his second generation guinea-pigs were so affected that the various litters included a heavy proportion of more or less malformed weaklings: and this very nearly represents the state of affairs in similar experiments, conducted with lead salts on the same animals, by Cole, Bachhuber, Weller and others. Pearl, on the contrary, found that the progeny of alcoholised fowls was measurably superior in several important respects to the offspring of untreated individuals. Almost in complete agreement with Pearl's work were the indications of Nice's white mice, and of Elderton and Pearson's studies on the influence of parental alcoholism in human beings. The scope of my projected experiments was greatly enlarged by the possibility that they would throw some light on the seeming discrepancy between the conclusions to be drawn from the various sets of work enumerated. Furthermore, as a group of animals far removed from the Mammalia or Aves was involved, the value of the work was greatly enhanced, whether its indications went to corroborate or to contradict any or all of the data just mentioned.

The questions then I set out to answer were

(1) Can I by the continued administration of ethyl alcohol so affect the germ cells of *Selenia bilunaria* as to repeat the conditions of Morgan's *Drosophila* cultures where hereditary variations appeared in very unusual numbers?

(2) Failing this, are the progeny of parents submitted to the action of the deleterious agent significantly affected by it?

(3) If not, do the actual phenomena encountered approximate to those observed in the case of Pearl's fowls and of Cole's white mice?

(4) And generally, what effects, somatically or otherwise, has the agent used on the treated individuals or their offspring?

As will be seen below, to the first and second questions I obtained a decidedly negative answer: in the case of the third, although not precisely the same as those with which comparison was desired, my results showed a considerable approach thereto in a broad fashion. The answers to the fourth will manifest themselves as the facts of the experiments are gradually unfolded.

II. TECHNIQUE EMPLOYED.

For several reasons, some personal and some arising from the war, after a short while it was deemed best that I should restrict myself to the use of ethyl alcohol throughout the work. At first sight, the difficulties attending the administration of this compound to lepidoptera seemed insuperable, and indeed are so if one wishes to employ the agent in the liquid form; these obstacles, however, quickly vanish if one considers the ease with which insects are affected at any stage of their life-histories by the vapours of chloroform, ether, hydrocyanic acid, sulphur dioxide and the like. The only trouble remaining is to devise some means of regulating the dose and of ensuring that its incidence is long enough. Here the advantages of utilising a Geometrid moth appertaining to such a hardy genus as *Selenia* are at once realised; a considerable number of their larvae can be reared in airtight cages without the slightest ill-effects manifesting themselves. If once the use of airtight cages is granted methods of generating constant and steady supplies of alcohol vapour so that ova, larvae, and pupae are always under its action are very simply designed. After a few preliminary experiments to determine the optimum quantity to be employed, one readily finds that by saturating a small sponge periodically with definite quantities of alcohol the atmosphere within the cage is kept uniformly charged with the fumes. Thus the creatures are never at any period of their growth and development free from its possible influence either on their germ or on their somatic cells. Only one precaution is necessary, and that is to suspend the sponge so that the larvae can never come into contact with the liquid used, which, in my case, was the ordinary 90% non-methylated ethyl alcohol of the chemist.

To secure the larvae experimented with, a healthy male and female *Selenia bilunaria* were taken from a brood once inbred and paired; the female in due course laid over a hundred fertile ova. In order to avoid overcrowding as far as possible, ninety of these were divided into two random batches of forty-five, one to be submitted to the alcohol regimen,

and the other to be enclosed in exactly the same kind of cage to serve as a control and therefore minus the alcohol. The value of the controls thus used was greatly increased by the fact that they were part of the same family as the treated lot.

The treatment was commenced soon after the ova were deposited, those to be alcoholised being shut up in airtight glass-topped tin boxes with a soaked sponge, and the others put into a similar box without alcohol.

The sponge was changed twice per day, once in the early morning and once at 8 P.M. Even after hatching, during their first instar, the larvae were kept in the same tins, but as they grew larger they were removed to bigger cages, and the dose of alcohol was simultaneously increased.

After pupation the treatment was still continued; be it ever so small, respiration takes place in the pupae, gametogenesis is being completed, and therefore the germ cells are capable of being influenced at a most critical time.

Throughout their early stages, both lots were provided with exactly the same food, hawthorn (*Crataegus oxyacantha*), and at the same time. Further, since the mortality (as will be seen below) was greater amongst the "alcoholists," whenever the cages were cleaned out the number of individuals in the control cage was reduced so as to correspond with those still alive in the other; this step was, of course, taken to eliminate possible errors arising from differences in the number of cubic inches of cage room allowed to each insect.

III. THE COURSE OF THE EXPERIMENT.

Although both sections of ova produced their full complement of larvae about May 4th, the effects of the alcohol were very soon visible when once the larvae commenced to feed, for almost immediately the mortality rate amongst the treated individuals became very great, no less than fourteen ($=31.1\%$) dying in the first instar. On the other hand, only two ($=4.4\%$) succumbed in the untreated controls—a number showing no sensible divergence from one's usual losses with first stage larvae. At this point, as shown in Table I, the controls were reduced to 31 so that once again the cages contained equal numbers. With the weeding out of these presumably very susceptible specimens the death rate in the intervals between the changing of the food and the cleansing of the cage slowed up, 13% , 14.9% and 17.3% being the

respective losses of the "alcoholists." The natural deaths amongst the untreated examples retained to act as controls were absolutely zero. However, as will be seen, one death is noted; this single death, as well as one of those marked with an asterisk in the other column, was due to the accidental crushing of the larvae by the twigs of the food plant.

TABLE I.

Date	Number of individuals treated with alcohol	Number of deaths amongst the treated larvae	Number of controls	Number of deaths amongst controls	Control reduced to
4/5/18	45	—	45	—	—
8/5/18	31	14	43	2	31
14/5/18	27	4	31	—	27
20/5/18	23	4	27	—	23
28/5/18	18	5*	22	1*	18
3/6/18	10	8	18	—	—
Pupation commences }					

One must be careful to explain that the two groups were not differentiated by the death rate alone; such a supposition does not agree with the facts. On May 18th, when the bulk of the controls cast their first skin, they did so a day and a half ahead of the most precocious member of the other lot, and the advantage thus gained was never lost; it was, on the contrary, increased so that on May 28th they were three days ahead, and when pupation commenced on June 3rd they span their cocoons six days earlier than their "drunken" brothers and sisters. In reality this latter interval should be reduced to five days because on May 30th, when all of the treated larvae had entered their last larval instar, the amount of alcohol administered was so excessive that all save three were stupefied and lay as if dead for 34 hours. The sponge was saturated at 6.30 A.M. and the larvae affected rendered unconscious by 7 A.M. When examined next day at 7 A.M., three had recovered, the rest following suit before 5 P.M. the same day.

Although possibly due to the deleterious effects of the ethyl alcohol on weaker and therefore smaller larvae with their consequent destruction, the retardation in growth was apparently linked up to some extent with the fact that the individuals exhibiting it attained a greater mean size than the majority of the controls did. In consequence, they produced correspondingly heavier pupae and larger imagines, thereby necessitating a longer period to reach their full growth.

Of the alcoholised caterpillars, ten, comprising seven males and three females, succeeded in spinning their cocoons and pupating therein; of the others, eighteen (including ten males and eight females) did likewise. The difference in sex ratios, in view of the small numbers

involved, has, in all probability, no definite import, although it is conceivable that the exaggeration of the disparity in the case of the treated lot points to some superior resistive powers on the part of the female zygotes to the effects of alcohol vapour.

Emergence commenced with the appearance of a control male on June 15th, to be followed at intervals by others until every single pupa had yielded its imago. The other group began to put in an appearance on June 18th, but of the ten pupae only seven, two males and five females, emerged. The remainder died shortly after pupation whilst the alcoholisation was yet being continued.

Thirty per cent. therefore died as pupae. Of those emerging two, one of each sex, were cripples. The male was a large strong insect and owed its crippling to accidental injury; the female, on the other hand, was a weakling and did not succeed in disentangling itself from the pupal integument. Had it not been freed artificially it would, undoubtedly, have perished within the pupal envelope.

As the imagines came out they were caged up in three separate muslin cages, the controls being paired *inter se*, and reciprocal pairings being made between them and opposite sexes of the "alcoholists." Owing to the fact that I never had a treated male and female out together, the fourth possible pairing, that between a male and a female treated example, proved unobtainable. When all of the insects had died, they were removed from the pairing cases and carefully measured with results seen in the appended table. It is perfectly evident that, in

TABLE II.

Wing Expanse	Alcoholised Individuals		Controls	
	Number of Males	Number of Females	Number of Males	Number of Females
34 mm.	—	—	3	—
35 mm.	—	—	2	—
36 mm.	1	—	3	5
37 mm.	—	1	—	2
38 mm.	—	1	—	1
39 mm.	—	2	—	2
Cripples	1	1	—	—
Total	1+1	4+1	8	10
Average wing expanse	36 mm.	38.25 mm.	35 mm.	37 mm.

both sexes, the insects which have been placed under the action of ethyl alcohol fumes during their growth and development periods are distinctly larger in size. In spite of the low numbers concerned, I feel certain that this is no chance effect, more especially when this superiority

in size over their untreated relatives is just what Pearl noted in his fowls.

In all cases the copulations proved successful, and an abundance of fertile eggs was laid. As a matter of fact every egg in the cages changed colour and thereby displayed its fertility. The exact total of those deposited in the cages by fertilised females, and in chip boxes by virgin females, is supplied with other essential facts in Table III.

TABLE III.

	Alcoholised Females		Control Females	
	Number of Individuals	Ova laid	Number of Individuals	Ova laid
Paired with control ♂ ♂ ...	2	248	5	603
Paired with alcoholised ♂ ♂	—	—	2	254
Not paired	2	274	3	386
Totals	4	522	10	1243
Average number of ova per female	—	130.5	—	124.3

Again as tested by the average number of ova per female, the untreated individuals were distinctly inferior to those which had undergone treatment with alcoholic fumes. Nor did the matter rest there; although every egg was fertile not every one hatched, and there were marked differences in the number of zygotes which perished in the shell, the mortality rate being obviously less when a treated imago figured as a parent. As with other statistical data, this is best set out in tabular form so that the precise values may be comprehended at a glance.

TABLE IV.

Pairing	Number of ova laid	Number which died in the shell	Percentage dying in the shell
Control ♂ ♂ × Control ♀ ♀ ...	603	47	7.8
Control ♂ ♂ × Alcoholised ♀ ♀ ...	248	5	2.01
Alcoholised ♂ ♂ × Control ♀ ♀ ...	254	7	2.7

Although the ova from the controls were laid in the four days following June 16th, those from the mating of an alcoholised male and a control female in the four days after June 19th, and those from the reciprocal cross after June 21st, the ova hatched in the reverse order; those from the last-named pairing emerging on July 1st, 2nd, and 3rd, those from the second lot on July 2nd, 3rd, and 4th, and those from the untreated controls spreading themselves out over the period between July 8th and July 14th, both days being included. Here we first see indicated that acceleration in development manifested by the offspring of treated parents at every stage.

As before, instead of rearing all the larvae, a random sample of fifty ova was chosen from each batch, and the resulting larvae fed up on hawthorn, the food plant of the parents. Unlike them, however, the three sets were reared in well-ventilated and well-lighted cages such as are employed in normal breeding operations. In the first four instars no deaths occurred in the crossings in which treated insects took part; even in the inbreeding of the controls the loss was small, the deaths in each skin being one, three, none, and four respectively.

When, however, the first larvae were ready to spin, these being derived from a pairing between a treated male and an untreated female, there were left fifty of that cross, forty-six of the reverse brood and thirty from the controls. In view of the fact that very great differences in speed of feeding up were now most strikingly displayed, it will be best to show the numbers spinning up and pupating each day in the form of a tabular diary. In this table (Table V) records are set out daily until August 22nd, when all of the earliest lot had spun, and for two day intervals subsequent to that.

TABLE V.

Date	Alcoholised Males × Control Females		Control Males × Alcoholised Females		Control Males × Control Females	
	Spun	Pupated	Spun	Pupated	Spun	Pupated
August 8th	3	—	—	—	—	—
August 9th	5	1	—	—	—	—
August 10th	11	2	5	—	—	—
August 11th	15	5	7	3	—	—
August 12th	21	12	10	6	1	—
August 13th	25	15	11	8	1	—
August 14th	31	21	13	10	2	1
August 15th	37	26	17	11	4	1
August 16th	40	32	18	13	6	2
August 17th	42	37	20	17	7	4
August 18th	44	40	22	18	9	5
August 19th	46	42	25	20	10	8
August 20th	50	44	28	21	14	9
August 21st	50	46	28	24	15	10
August 22nd	50	50	30	27	17	14
August 23rd—25th	50	50	30	28	19	17
August 25th—27th	50	50	32	30	23	20
August 27th—29th	50	50	32	30	24	21
August 29th—31st	50	50	36	32	25	21
August 31st—September 2nd	50	50	37	32	25	24
September 2nd—4th	50	50	41	36	25	25
September 4th—6th	50	50	41	41	28	26
September 6th—8th	50	50	41	41	28	27

From the above figures it is very clear that the total loss in the cross between an alcoholised male and a control female is zero, that in the reciprocal cross is nine ($= 18\%$), and amongst the controls twenty-

three ($= 46\%$). This result is the more significant when one recollects that all three lots were descended from the same great-grandparents and grandparents and had thus been twice inbred—a fact explaining the low vitality of the controls which the administration of alcohol to the parents had apparently surmounted in the case of the others.

Nor was the superiority where the parentage included an alcoholised individual confined solely to the low death rate; it was exhibited in the general rapidity of feeding up and growth as can readily be gleaned from the facts and data given. Even more remarkable was the fact that, despite the greater rate of growth, the members of the same two broods attained a much greater size in both sexes. To enable this to be grasped with facility use is once more made of a table. Profiting from my experience gained with their parents, when cripples were not available for measurement, in compiling Table VI, instead of taking the imaginal wing expanse I extracted the pupae from their cocoons, weighed them and utilised the pupal weights which appear, classified according to brood and sex, below.

TABLE VI.

Brood	Males			Females		
	Number of Individuals	Weight	Average Weight	Number of Individuals	Weight	Average Weight
Alcoholised ♂ ♂ × Control ♀ ♀	23	3.79 grms	.165 grm	27	5.25 grms	.194 grm
Control ♂ ♂ × Alcoholised ♀ ♀	18	2.55 grms	.142 grm	23	4.07 grms	.177 grm
Control ♂ ♂ × Control ♀ ♀	12	1.44 grms	.12 grm	15	2.02 grms	.135 grm

The insects began to come out on August 24th when a male proceeding from the pairings in which the alcoholised male took part appeared. Others followed by degrees until August 27th when four males and one female had been bred. On that date two females representing the reverse cross joined company. The first control, a male, only emerged on September 1st when the other two batches were nearly all out: in fact, two-thirds of the treated male × untreated female lot and one-half of the other were already on the setting boards. Nevertheless, within the ensuing seven days, every insect had been reared save for one control which had died in the cocoon as an unchanged larva.

Just as one might anticipate from their greater pupal weight, the imagines from the alcoholised series were much the larger insects in both sexes. In addition to this, they displayed their robustness in a very

noteworthy and unexpected way. I mentioned above that the race of *Selenia bilunaria* with which we are concerned was double brooded, but I did not then indicate that it was seasonally dimorphic; such, nevertheless, is the case. The spring brood consists of larger, heavily and richly pigmented individuals, whilst the summer¹ brood is smaller and very weakly provided with pigment when compared with its spring relatives. This difference is caused directly by the longer growth period of normal autumnal larvae (which produce the spring imagines) lending itself to the production of larger, sturdier pupae. In spite of the circumstance that the two broods including a treated example in their parentage actually fed up more quickly than the controls, they exhibit in most of their members a facies exceedingly close to that of an ordinary spring brood insect for they possess all its depth and richness of coloration—an occurrence depending immediately on their superior size and vitality. The controls, on the contrary, are smallish specimens differing in no respect from the washed out looking July brood which Haworth called *juliaria*.

In the progeny of Stockard's alcoholised guinea-pigs one of the most striking things was the development of individuals malformed in respect to various organs, but more especially in the eyes. In the case of these insects now being discussed, no such abnormalities presented themselves; all of the imagines, no matter what their parentage, were equally perfect in all of their organs.

The sex ratios in all cases call for no comment although in all, exactly as in the broods from which their parents were derived, an excess of females appears.

IV. DISCUSSION OF THE RESULTS.

To summarise the results we may state:

- (1) The offspring of treated parents neither included monstrosities nor displayed even slight abnormalities.
- (2) No new hereditary variations occurred.
- (3) The survivors of the treated batch grew, on the average, decidedly larger than their untreated relatives. This may have arisen simply from the elimination of the weaker individuals by the deleterious agent employed, or it may have been caused by some physiological action of the ethyl alcohol. In any case it agrees with Pearl's experience with

¹ Should, by chance, three broods appear in one season the third resembles, in every respect, the second or normal summer brood.

his poultry; since his mortality rate was negligible, in all probability we have to look to both of the suggested causes for a correct explanation in this instance.

(4) The progeny of the treated batches was greatly superior in several respects to that of the untreated controls.

(5) The offspring from the cross between a treated male and an untreated female was superior to that of the reverse mating to much the same degree as the latter was better than the controls.

(6) The superiority of the broods originating with treated parents was exhibited in:

(a) The smaller percentage of embryos perishing in the shell, the exact figures being 2.01% and 2.9% against 7.8%.

(b) The quicker development of the embryo.

(c) The speedier rate of feeding up in the larva.

(d) The lower rate of larva mortality, 0%, and 18% against 46%.

(e) The greater mean weight of the male pupa.

(f) The greater mean weight of the female pupa.

(g) The quicker development of the imago and its earlier appearance.

(h) Its richer pigmentation.

(7) In sex ratio and in the percentage of fertile eggs no differences were perceptible.

Except, therefore, in the percentage of fertile eggs and in characters only capable of manifestation in the lepidoptera, my data in the main tend to point in the same direction as those of Pearl.

To explain the results I think we have to look to the cumulative effects of selection of two different types acting at different stages: (I) the first of these acts on the parental zygotes when the alcohol weeds out the weaker insects and preserves the stronger to perpetuate their race; (II) the second works directly on the germ cells of these selected, stronger, and sturdier survivors.

The first point needs little or no elaboration as it is almost self-explanatory; still perhaps a few words are necessary. As only insects superior in size and vigour survived the action of the alcohol to act as parents it is a fair inference that their gametes would be of a superior type. Of necessity, then, zygotes into whose composition these gametes enter would manifest greater strength and vitality. Thus if the qualities in which the broods from alcoholised parents excel depend in part at least on parental excellence then such broods would

commence life with a weighty advantage in that respect over the progeny of untreated lots which have undergone no similar process of selection.

The second part demands more consideration. Throughout the lives of the parent insects their germ cells have been constantly liable to injury from the alcohol vapour. Now, as Pearl suggested, almost certainly the germ cells will not be alike in resistive powers, and therefore the alcohol will not affect all in a similar manner. Merely considering the germ cells as acted on by a medium dose of alcohol, there are three possibilities: (1) that weaker germ cells can be destroyed or rendered wholly ineffective; (2) that more vigorous ones can be weakened; (3) that germ cells of the Grade I type can be so refractory as to be quite unaffected. Clearly, too, by increasing the dose of alcohol we could progressively eliminate all three classes. Supposing that the amount employed in the present experiment was just sufficient to destroy the feeblar and medium members of the germ cell population, what would that lead to? It would only leave the most superior to continue the race. If, as seems undoubtedly the case, an "A1" germ cell population guarantees "A1" zygotes, then the subsequent generation should all be "Grade 1." And this exactly fits in with the insects reared from the cross between a treated male and an untreated female. Every single imago of this parentage was vastly superior to all controls. Therefore selection, in the first place of first quality parents producing the finest type of germ cells, followed by intense selection amongst these chosen germ cells themselves, affords an adequate explanation of the circumstances of this brood.

If, however, the weaker cells alone are inactivated, and the stronger and medium left, the former absolutely uninjured and the latter affected in varying degrees, then the zygotes resulting from gametes so differentiated would be of two grades: (1) composed of sturdy individuals much the same as what we have just considered in the case of the previous mating; (2) including distinctly less vigorous examples. This agreed precisely with the state of affairs in the insects raised from the pairing of an untreated control male and an alcoholised female.

From the foregoing it will be perfectly obvious that, despite the fact that both alcoholised males and females received the same dose of ethyl alcohol and for the same time, the conditions of the broods from reciprocal crosses in which these insects took part were utterly dissimilar. It therefore follows that, as far as these insects are concerned, the gametes of the female are much less susceptible to

alcoholic selection than those of the male—which is entirely in accord with one's expectations. after giving due attention to the protection afforded to the gametes of the female for such a prolonged period of their existence.

That the whole of the zygotes yielded by the mating of an alcoholised male and a control female were substantially alike, and that those from the reverse pairing were not so, was actually capable of proof. When exactly one-half of the first-named lot had pupated they were weighed, as were also the representatives of the other lot then in pupa. A similar course was then adopted when the other half had changed. It was then discovered that, whereas the two halves of the former agreed in the mean weights of the male and female pupae, the second (as well as the third) portion of the other cross was clearly inferior to the first as will be readily perceived from the following table:

TABLE VII.

	Alcoholised Males × Control Females		Control Males × Alcoholised Females	
	Average Male Weight	Average Female Weight	Average Male Weight	Average Female Weight
First Portion, August 15th	·164 grm	·191 grm	·163 grm	·186 grm
Second Portion, August 23rd	·195 grm	·198 grm	·141 grm	·177 grm
Third Portion, } September 6th }	All emerged } by Aug. 23rd }	All emerged } by Aug. 23rd }	·126 grm	·171 grm

If this hypothesis is in very truth the scheme that forms the ground-work of the observed facts then my insects, when the requisite allowance is made for the additional phenomenon of parental selection, fall in line with Pearl's poultry and not with Stockard's guinea-pigs, for in the latter the destruction and injury of the germ cells of treated parents had proceeded much further than in the germ cells of my *Selenia bilunaria* males. This, too, serves to demonstrate why neither Pearl nor I reared malformed progeny while Stockard did so.

In conclusion, only one more point needs explaining, and that is the great feebleness of the controls. This is quite consistent with one's expectations. The genus *Selenia*, like many (but not all) lepidopterous genera, is extremely impatient of inbreeding and soon dwindles in size, productivity, and vigour under its influence. That parental and gametic selection was able to counteract this only emphasises further the lessons of the experiment, for it must not be forgotten that controls and "alcoholists" alike had the same great-grandparents and grandparents, and that, necessarily, their immediate parents were brothers and sisters.

LITERATURE REFERRED TO.

- COLE and BACHHUBER. "The effect of lead on the germ cells of the male rabbit and fowl as indicated by their progeny." *Proc. Soc. Exper. Biol. Med.* Vol. XII. pp. 24—29. (1914.)
- ELDERTON and PEARSON. "A first study of the influence of parental alcoholism on the physique and ability of the offspring." *Eugenic Lab. Mem.* Vol. x. pp. 1—46. (1910.)
- NICE. "Comparative studies on the effects of alcohol, nicotine, tobacco smoke and caffeine on white mice. I. Effects on reproduction and growth." *Journ. Exper. Zool.* Vol. XII. pp. 133—152. (1912.)
- PEARL. "Some effects of the continued administration of alcohol to the domestic fowl, with special reference to the progeny." *Proc. Nat. Acad. Sci.* Vol. II. pp. 675—683. (1916.)
- . "The experimental modification of germ cells." Parts I, II, and III. *Journ. Exper. Zool.* Vol. XXII. Nos. 1 and 2. (1917.)
- STOCKARD. "An experimental study of racial degeneration in mammals treated with alcohol." *Arch. Intern. Med.* Vol. x. pp. 369—398. (1912.)
- . "The effects on the offspring of intoxicating the male parent and the transmission of the defects to subsequent generations." *Amer. Nat.* Vol. XLVII. pp. 641—682. (1913.)
- . "A study of further generations of mammals from ancestors treated with alcohol." *Proc. Soc. Exper. Med. Biol.* Vol. XI. pp. 136—139. (1914.)
- WELLER. "The blastophthoric effect of chronic lead poisoning." *Journ. Med. Resear.* Vol. XXVIII. pp. 271—293. (1915.)

INHERITANCE OF WING COLOUR IN LEPIDOPTERA.

II. MELANISM IN *TEPHROSIA CONSONARIA* (VAR. *NIGRA* BANKES).

By H. ONSLOW.

(With Plate II.)

IN a previous communication¹ the writer described a case of variation in *Abraaxas grossulariata*, involving a change in the pigment of the normal ground-colour, from white to yellow. The present note deals with an example of melanism, one of the most remarkable phenomena of variation, because of its recent rapid progress in England and on the Continent. In an article by Bowater² evidence is given, showing that the melanic variety of *Odontoptera bidentata*, though somewhat variable in intensity, is dominant to the ordinary pale form. The same author summarises most of the available breeding experiments carried out with other melanic varieties. Though the published evidence is scanty and often obscure, he concludes that melanism may sometimes be recessive, but that it is more frequently dominant to the type form.

Melanism is widely distributed among the Geometridae, and is especially frequent in the sub-family Boarmiinae, a great number of which have melanic forms. Among other species the following are at present being investigated, and the results will shortly be published: *Boarmia abietaria*, *Boarmia consortaria*, and *Tephrosia consonaria*. The last-mentioned species forms the subject of the present communication.

The experiments were planned so that the inheritance of melanism might be studied on a much larger scale than is possible by compiling the published accounts of chance experiments. The primary object, however, was to select two species, preferably closely related, in one of

¹ Onslow, H., *Journal of Genetics*, Vol. VIII. No. 4, p. 209, Sept. 1919.

² Bowater, W., *Journal of Genetics*, Vol. III. p. 299, April 1914.

which melanism was dominant, and in the other recessive to the type form. It was then proposed to carry out a chemical study of the metabolism of the adult larvae and the pupae of these two species, in the hope that some light might be thrown on the mechanism responsible for such dissimilar phenomena, and possibly on the wider problem of the chemical aspect of the laws of heredity.

The history of the melanic form of *T. consonaria* (the Square Spot) is not without interest, the most important point being, that it has, as far as I can ascertain, only been found in a single locality, and one moreover which is very far removed from the traditional home of melanic varieties, the Black Country.

In 1892, E. Goodwin first found this form, subsequently described as var. *nigra*¹, in an oak wood at Watlington near Maidstone, and it is said to have occurred there every year up to the present. The locality is referred to by various authors as North Kent and West Kent, but I understand the same oak wood is intended. The following interesting details are given².

Ova obtained from normal females in the affected district yielded about 10% melanic; ova from black females yielded from 30% to 75% melanic, averaging about 50%. Black ♀ × black ♂ gave 38 melanic and 4 typical.

Since it is clear from the experiments hereafter recorded that var. *nigra* is a simple Mendelian dominant, these figures require some explanation, because at first sight it might be thought that since normal females give 10% melanic, the black form is recessive. If var. *nigra* is dominant, the least number of melanics that could be produced from a type ♀ mated to a melanic ♂ is 50%, supposing the male to be heterozygous. The only explanation, therefore, is that the females were fertilized by type as well as by melanic males. It is to be expected that black females should give 75% melanic, if both parents were heterozygous. Black females giving 30% melanic might possibly be heterozygotes paired to a normal male, in which case the expectation would be 50%, but it seems more probable that here again the females have paired at least twice, that is to say, to a melanic and to a normal male, and that the ova have been fertilized by the spermatozoa of both, which have become mixed in the spermatheca. That males will pair several times is well known. In 1918, for instance, I had two *Abraxas grossulariata* males, var. *varleyata*. One of them paired no less than 9 times,

¹ Bankes, E. R., *Ent. Mag.*, London, Vol. xli. p. 89, 1905.

² Doncaster, L., *Ent. Record*, Vol. xviii. p. 223, 1906.

though none of the pairings were fertile. The other paired 5 times, the first two pairings being sterile and the last three pairings fertile. The female does not often pair twice, but I have more than once seen this occur, though in breeding the female is usually isolated as soon as fertilized.

The appearance of the type and melanic insects in the following experiments is shown on Plate II. The melanic females are a deep black (figs. 4 and 5), the melanic male is rather browner (fig. 6), the antennae being very slightly pectinated. In both sexes the veins are traced in deeper black, especially noticeable when freshly emerged. The characteristic "square spot" between the second and third submarginal lines is naturally invisible, but usually a pale wedge-shaped marking, more pronounced in the female than in the male, makes its appearance just at the inner margin of the square spot. The only other white marking is a faint wavy line on all four wings, which in the type insect is situated at the outer margin of the square spot. All the type insects, extracted from melanics, were greyer and less ochreous than is usually the case with wild individuals. This is especially noticeable in the female (figs. 1 and 2), in which the speckling is grey and many of the markings black. The males tend to be browner and the markings are slightly blurred and indistinct. Fig. 3 represents one of the darker males, but none of these in any way approached the melanic series.

I am indebted to Mr L. W. Newman for procuring me the melanic ova for experimental purposes, and it is with his permission that I publish the following details. In June 1914 a wild melanic ♀ and several type ♂♂ were taken in the oak wood at Wateringbury. After capture, the ♀ deposited ova, and from the larvae which resulted, three melanic insects emerged in 1915, 2 ♀♀ and 1 ♂. With these, three fertile pairings were obtained, the melanic ♂ pairing twice; the larvae all did well and the following insects emerged in 1916:

Family	Parentage		Imagines	
	Female	Male	Melanic	Type
'15 C	<i>M</i>	× <i>M</i>	95	—
'15 A	<i>M</i>	× <i>T</i>	89	1
'15 B	<i>T</i>	× <i>M</i>	132	3
Totals	316	4

The larvae were not kept under experimental conditions, and the four type insects recorded were possibly introduced by accident. The fact that no type insects appeared in families '15 A and '15 B suggests that the melanic parents were homozygous for the melanic factor. If this

were so, the original captured ♀ must have paired with a wild melanic ♂.

Fig. 1 gives a schematic representation of the history of the strain, showing how the 1918 families were descended from the original stock. Each family is labelled with a distinctive letter, and the year in which the ova were laid.

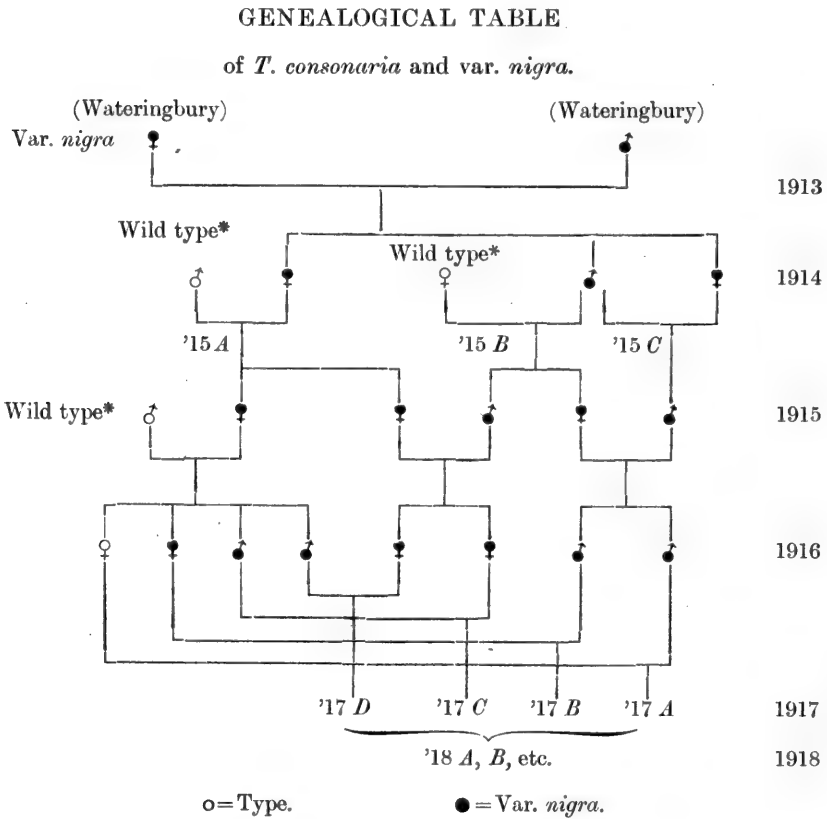


Fig. 1. Schematic representation of the relationship of the 1918 families of *T. consonaria* and var. *nigra* to the original stock.

* Captured in the same oak wood at Wateringbury.

In 1918 a sufficient number of insects were obtained to make all the necessary pairings. The larvae are very hardy and remained almost free from dysentery and other diseases. The species seems to withstand a certain amount of inbreeding without showing any ill effects. The

eggs were kept in metal boxes, in which the young larvae were allowed to remain a fortnight. They were then transferred to the ordinary type of glass breeding cylinders. Since the larvae will only eat the tenderest oak leaves, sycamore (*Acer pseudoplatanus*) was largely used towards the end of June, when the larvae commence "feeding-up." It is, however, sometimes possible to get a supply of adventitious oak shoots, from trees which have been stripped some time previously by any small Geometer. The pupae were allowed to remain in the leaf-mould and sand in which they pupated, until the following spring. Over one thousand insects emerged in 1919, and are shown in the following tables. When the type insects used for pairings were not extracted from melanics, they came from wild pupae collected in Buckinghamshire.

The result of pairing two melanics, at least one of which was homozygous, was as follows:

$$\text{Melanic} \times \text{Melanic} [DD \times DD(R)].$$

Family	Bred by	Imagines					
		Melanic			Type		
		Male	Female	Totals	Male	Female	Totals
'15 C	L. W. Newman	—	—	95	—	—	—
'18 M	H. O. ...	47	51	98	—	—	—
Total		193	—	—	—

The result of pairing two melanics, both of which were heterozygous, was as follows:

$$\text{Melanic} \times \text{Melanic} [DR \times DR].$$

Family	Bred by	Imagines					
		Melanic			Type		
		Male	Female	Totals	Male	Female	Totals
'17 B	H. O. ...	9	7	16	3	4	7
'17 C	H. O. ...	3	5	8	1	1	2
'17 D	H. O. ...	5	5	10	2	—	2
'18 L	H. O. ...	30	22	52	13	11	24
'18 R	H. O. ...	13	12	25	3	1	4
Totals		111 (74%)	39 (26%)		
Expectation		112.5			37.5		

The percentage of types to melanics is very close to the expected ratio 75 : 25.

The result of mating homozygous melanics to types should give nothing but melanics. The result was, however, as follows:

*Melanism in Tephrosia consonaria**Melanic* × *Type* [*DD* × *RR*].

Family	Parentage		Bred by	Imagines					
				Melanic			Type		
	Female	Male		Male	Female	Totals	Male	Female	Totals
'15 <i>A</i>	<i>M</i>	× <i>T</i>	L. W. Newman	—	—	89	—	—	1
'15 <i>B</i>	<i>T</i>	× <i>M</i>	L. W. Newman	—	—	132	—	—	3
'17 <i>A</i>	<i>T</i>	× <i>M</i>	H. O. ...	3	7	10	—	—	—
'18 <i>B</i>	<i>M</i>	× <i>T</i>	H. O. ...	54	27	81	1	—	1
'18 <i>K</i>	<i>T</i>	× <i>M</i>	H. O. ...	3	4	7	—	—	—
'18 <i>T</i>	<i>M</i>	× <i>T</i>	H. O. ...	9	6	15	—	—	—
Totals				334	5		

Five types are recorded. Four of them occurred in the pairings made by Mr L. W. Newman, that is to say, before the larvae were kept under experimental conditions, and had possibly strayed from some other source. The percentage of types included in the later families is less than 1 per cent.

The type parents of families '15 *A* and '15 *B* above were captured in the same wood as the original melanic ♀. The type parents of '17 *A* and '18 *T* were extracted from melanics. The type parents of '18 *B* and '18 *K* were wild insects from pupae collected in Buckinghamshire.

The result of pairing heterozygous melanics with types, either way, gives almost exactly 50% melanic and 50% type, as follows:

Melanic × *Type* [*DR* × *RR*].

Family	Parentage		Bred by	Imagines					
				Melanic			Type		
	Female	Male		Male	Female	Totals	Male	Female	Totals
'18 <i>A</i>	<i>M</i>	× <i>T</i>	H. O.	43	27	70	32	38	70
'18 <i>C</i>	<i>T</i>	× <i>M</i>	H. O.	15	17	32	12	17	29
'18 <i>G</i>	<i>M</i>	× <i>T</i>	H. O.	13	12	25	10	18	28
'18 <i>H</i>	<i>M</i>	× <i>T</i>	H. O.	7	8	15	9	9	18
'18 <i>I</i>	<i>T</i>	× <i>M</i>	H. O.	6	3	9	6	10	16
'18 <i>N</i>	<i>M</i>	× <i>T</i>	H. O.	12	8	20	2	8	10
'18 <i>Q</i>	<i>T</i>	× <i>M</i>	H. O.	4	9	13	3	8	11
'18 <i>V</i>	<i>M</i>	× <i>T</i>	H. O.	13	20	33	4	22	26
Totals	217 (51%)	208 (49%)		
Expectation	212.5	212.5		

The type parents of families '18 *A*, *C*, *G*, *H*, *I*, *V* were wild type insects. Those of families '18 *N* and '18 *Q* were types extracted from melanics. In this and the previous cases reciprocal crossings were obtained, but it appears to make no difference which parent was melanic and which type. The sexes have also been separated in each family, but the proportions appear to have no significance.

The result of pairing two types together is to produce nothing but types, whether the parents are extracted from melanics or not, as follows:

$$\textit{Type} \times \textit{Type} [RR \times RR].$$

Family	Bred by	Imagines					
		Melanic			Type		
		Male	Female	Totals	Male	Female	Totals
'17 <i>E</i>	H. O.	—	—	—	4	8	12
'18 <i>F</i>	H. O.	—	—	—	58	53	111
'18 <i>O</i>	H. O.	1	1	2	50	40	90
'18 <i>P</i>	H. O.	—	—	—	23	33	56
Totals ...				2	269		

In family '17 *E* both parents were wild types; in family '18 *F* the ♂ parent was an extracted type; in families '18 *O* and '18 *P* the ♀ parents were extracted types. In this case again less than 1% of melanic insects have been included accidentally¹. The eggs are so exceedingly small that it is extremely easy to transfer one inadvertently to the wrong box in the hairs of the brush.

The evidence from the above experiments, which include over 1,400 insects, shows that the melanic variety of *T. consonaria* behaves as a simple Mendelian dominant with regard to the pale type form. Moreover the melanics are a perfectly definite class and show no tendency to vary in either direction.

In conclusion I should like to express my thanks to Mr L. W. Newman for supplying me with the material, and some of the details mentioned above, and to Miss Helen Moodie for her care of the larvae, to which the size of the families is entirely due.

¹ (Note added Nov. 25, 1919.) Professor J. W. H. Harrison, whose work on this species will be published shortly, tells me he has had occasional melanics from pure type pairings, and *vice versa*, which could not be attributed to accident, as they occurred in single test broods. With the possible exception of the 1915 broods (p. 55) I have been unable to exclude the possibility of error.

DESCRIPTION OF PLATE II.

Tephrosia consonaria and var. *nigra*. $1\frac{1}{2}$ times natural size.

1. *T. consonaria* ♀. Type, bred from var. *nigra*.
2. *T. consonaria* ♀. Type, bred from var. *nigra*.
3. *T. consonaria* ♂. Type, from *T. consonaria* ♀ × var. *nigra* ♂.
4. Var. *nigra* ♀.
5. Var. *nigra* ♀.
6. Var. *nigra* ♂.



1



4



2



5



3



6



RACIAL STUDIES IN FISHES. III. DIALLEL CROSSINGS WITH TROUT (*SALMO TRUTTA* L.).

By JOHS. SCHMIDT, D.Sc.

*Director of the Carlsberg Physiological Laboratory,
Copenhagen, Denmark.*

IN previous papers I have summarized the results of some investigations carried out in the Carlsberg Laboratory in Copenhagen with two viviparous species of fish belonging to the genera *Zoarcetes* and *Lebistes* (*Journal of Genetics*, Vol. VII, 1918, and Vol. VIII, 1919).

It was shown there that the number of vertebrae, fin-rays, etc.—i.e. so-called “racial” characters—are certainly capable of environmental influences, but still primarily determined by internal, hereditary factors¹.

I shall here give an account of some experiments with a third, oviparous, species of fish, whose conditions of reproduction are very favourable to experimental investigation. With this species it has therefore easily been possible to confirm the results previously arrived at with the two viviparous species, and for the same reason it may, presumably, with advantage be used to elucidate generally the difficult questions of the genetic behaviour of quantitative characters.

This species with which I have especially worked is the ordinary trout (*Salmo trutta* L.), with which in this country artificial hatching, applied with a greatly developed technique, is carried on on a large scale.

With a number of trout I perform what I have called *diallel* crossings. It consists in this that *each female is paired with each male*. If for instance there are a males and b females, $a \times b$ offspring-combinations will arise which are all of them different and for which the average values of the character in question are determined by observation. As

¹ Professor R. C. Punnett arrived at a similar result by a statistical examination of the viviparous shark *Spinax niger*, as is evident from an interesting memoir: “Merism and Sex in *Spinax niger*” (*Biometrika*, Vol. III, No. 4, 1904), which was unknown to me, but of which Prof. Punnett has been kind enough to send me a copy.

to particulars of the diallel crossings I refer to a previously published memoir¹.

The experiment here described was carried out during the winter 1918—1919. On the 10th of December 1918 diallel crossings were performed with 7 specimens of trout, viz. 3 males indicated by *x*, *y* and *z* and 4 females (*a*, *b*, *c*, *d*). From this arose 12 different offspring-combinations. The larvae hatched out about April 10 and were killed and preserved on May 15, 1919, when the yolk-sac was just resorbed. The experiment was carried out in the south of Jutland at the "Kongea" (Kingsriver) by Mr A. Christiansen. The countings were made by Mr Vilh. Ege, M.Sc.

The character examined was the number of vertebrae, of which the parents exhibited the following:

$$\begin{array}{cccc} \sigma & x & y & z \\ & 59 & 60 & 59, \\ & a & b & c \\ & 61 & 59 & 57 & d & 58. \end{array}$$

For each offspring-combination 50 specimens were examined, in which the number of vertebrae were counted under the microscope, and after this the *average* was calculated for the 50 specimens of each combination.

With notations that indicate an offspring-combination by the symbols of the parents the results were as follows:

TABLE I.

Average number of Vertebrae in Offspring (50 specimens of each combination).

<i>xa</i>	<i>xb</i>	<i>xc</i>	<i>xd</i>
61.14	59.06	58.29	59.03
60.0	59.0	58.0	58.5
<i>ya</i>	<i>yb</i>	<i>yc</i>	<i>yd</i>
61.35	59.22	58.59	59.28
60.5	59.5	58.5	59.0
<i>za</i>	<i>zb</i>	<i>zc</i>	<i>zd</i>
60.65	58.48	57.90	58.55
60.0	59.0	58.0	58.5

In this table the offspring-combinations of a horizontal row have the same father, and those of a vertical row have the same mother.

¹ Johs. Schmidt: "La valeur de l'individu à titre de générateur, appréciée suivant la méthode du Croisement diallèle" (*Comptes Rendus des Travaux du Laboratoire Carlsberg*, Vol. xiv, No. 6, Copenhagen, 1919).

The first value under each symbol is the average of the observed numbers of vertebrae in the offspring, the second (in italics) the mean of the parental numbers of vertebrae.

A comparison of the two values shows that in some cases the averages of the offspring and of the parents coincide closely or rather closely (e.g. *xb* and *zd*), while in other cases they differ very much (e.g. *xa*). In other words, there does not appear to be a simple rule connecting the number of vertebrae in the offspring with that in the parents. A closer examination of the values makes it probable that a rule nevertheless exists.

By my previous investigations it has been proved that offspring of the same parents developed under unequal environmental conditions may differ in the number of organs, such as fin-rays, vertebrae, etc. From this it follows that it is necessary to distinguish between the realized, purely *personal* value of a given individual trout—this value would have been a different one, if the individual in question were developed in different environments—and the *generative* value of the same individual, and that is the value which it imparts to its offspring.

It is thus beyond doubt that an individual may have a generative value different from the personal value, and it is possible, nay probable, that in this point we find the cause of the apparent discrepancy above mentioned between the average of the parents and that of the offspring.

At any rate we shall start from the assumption that the average for a number of offspring-individuals closely coincides with the average of the generative values of the parents and inquire whether this supposition does agree with the values arrived at in the experiment. Expressed by formulae our assumption is then that

$$\frac{x+a}{2} = 61.14, \quad \frac{y+a}{2} = 61.35, \quad \frac{z+a}{2} = 60.65,$$

where *x*, *y*, *z*, *a*, etc. indicate the generative value of the individual in question.

By summation of the equations containing *x* (corresponding to the first horizontal line of Table I) we find

$$2x + \frac{1}{2}(a+b+c+d) = 237.52.$$

In the same way we find, by summation of the equations containing *y* and *z* respectively,

$$2y + \frac{1}{2}(a+b+c+d) = 238.44,$$

and

$$2z + \frac{1}{2}(a+b+c+d) = 235.58, \text{ etc.}$$

By subtraction we obtain from these equations

$$\begin{aligned}x - z &= 0.97, \\ y - z &= 1.43, \\ a - b &= 4.253, \\ a - c &= 5.573, \\ a - d &= 4.187.\end{aligned}$$

Provided our assumption be correct, we have then determined the differences between the generative values of the various individuals, males in one group and females in another.

If we want a measure for the generative values of the single individuals we must fix a starting-point. Let us assume that the generative value of the male y coincides with the personal value of the same specimen, viz. 60, in other words, let us refer all the individuals to those environmental conditions under which the genotype y realizes 60 vertebrae. We then find

$$\begin{aligned}x &= 59.54, \\ y &= 60, \\ z &= 58.57.\end{aligned}$$

To find the generative values of the females, e.g. of a , we introduce those values in the equations

$$\frac{x + a}{2} = 61.14, \quad \frac{y + a}{2} = 61.35, \quad \frac{z + a}{2} = 60.65,$$

and we obtain thus three values for a , viz.

$$\begin{aligned}a_x &= 62.74, \\ a_y &= 62.70, \\ a_z &= 62.73,\end{aligned}$$

the average of which is 62.723.

Proceeding in the same way with the other females we find

$$\begin{aligned}a &= 62.723, \\ b &= 58.470, \\ c &= 57.150, \\ d &= 58.537,\end{aligned}$$

with which we have determined the generative values of all the individuals.

We are now prepared to test our theory by comparing the theoretical values with those obtained by examination of the offspring. By introducing the generative values above calculated into the expressions $\frac{x+a}{2}$, $\frac{x+b}{2}$, etc. we arrive at a *theoretical* value which we want to compare with the average *observed* in the offspring. The results are contained in Table II.

TABLE II.

Comparison of Observed and Calculated Values in the Offspring.

Combination	Observed	Calculated	Difference	Probable Error of observed values
<i>xa</i>	61.14	61.13	+ 0.01	0.060
<i>xb</i>	59.06	59.01	+ 0.05	0.040
<i>xc</i>	58.29	58.35	- 0.06	0.039
<i>xd</i>	59.03	59.04	- 0.01	0.041
<i>ya</i>	61.35	61.36	- 0.01	0.056
<i>yb</i>	59.22	59.24	- 0.02	0.048
<i>yc</i>	58.59	58.58	+ 0.01	0.048
<i>yd</i>	59.28	59.27	+ 0.01	0.051
<i>za</i>	60.65	60.65	0.00	0.054
<i>zb</i>	58.48	58.52	- 0.04	0.049
<i>zc</i>	57.90	57.86	+ 0.04	0.044
<i>zd</i>	58.55	58.55	0.00	0.048

It will be seen that the agreement between the observed and the calculated values is extremely good, also when it is kept in mind that we have had 6—that is 7 minus 1—values of which to dispose freely for calculating the theoretical values. The theory does thus obtain the utmost support obtainable from these experiments.

In Table III are given the personal values of the 7 trout together with their generative values calculated above from the investigation of the offspring. As will be remembered, they were determined on the supposition that all the individuals were referred to environmental conditions under which the genotype *y* realizes 60 vertebrae.

TABLE III.

The Personal and the Generative Values of the 7 Experimental Trout.

Symbols	Personal Values	Generative Values
<i>x</i>	59	59.54
<i>y</i>	60	60 (assumed)
<i>z</i>	59	58.57
<i>a</i>	61	62.72
<i>b</i>	59	58.47
<i>c</i>	57	57.15
<i>d</i>	58	58.54

The table shows that from the personal value of an individual few or no conclusions can be drawn with regard to the generative value. While x and z exhibit the same personal value, viz. 59, the generative value of x is about 1 greater than that of z . On the other hand b and d are nearly identical as regards generative value whereas one of them has realized 1 vertebra more than the other. Between the personal values of a and b and of b and c the differences are in both cases 2, but the differences of the generative values are 4.25 and 1.32 respectively, etc., etc.

CONCLUDING REMARKS.

It was mentioned in the introduction that the previous investigations with other species of fish had shown that the number of vertebrae, etc. certainly was influenced by the environment, but still primarily was determined by inheritance. As regards the trout-experiments the fact alone that it has been possible to work, apparently successfully, with the notions *personal* and *generative* values and fix them numerically involves a confirmation of the results of the previous investigations.

Of greater general or rather practical importance is the question, whether the experience gained from the trout-experiments, so favourable in regard to technique, can be made use of in the study of other quantitative characters of greater practical importance in animals and plants. The greater the difference between personal and generative value of an individual the greater the importance, e.g. for the breeder, of a basis which enables him to select animals to breed from, by a systematic valuation of their generative values.

In such cases where it appears that some important quantitative character is inherited in the same way as the number of vertebrae in the trout it may be hoped that the method of diallel crossings with the determination of the average values of the offspring-combinations will prove to be of use.

In most cases, at any rate in the animal kingdom, it will probably be difficult to obtain so great an accuracy in the determination of the generative value of an individual as with the trout. This species is distinguished by a great fecundity and by the great ease and accuracy with which the quantitative character in question could be measured. However, even a less accurate determination would often be of importance, e.g. if the question was of a comparative valuation of some personally identical individuals of a species in which the character

studied is highly dependent on environment¹. I am continuing the work with the method of diallel crossings applied to various species and am especially prepared to test the theory of the method through a study of the subsequent generations.

¹ It is not here the place to discuss at length the difficulties of technical or other nature which the application of the method of diallel crossings may present. I shall only mention that a small fecundity in a species does not necessarily exclude the application of the method, when the character in question may be accurately measured, and, altogether, is inherited in the same way as the character dealt with here. Let us imagine the least possible fecundity, i.e. a brood of one, and let us suppose that we want to compare the generative values of the two males x and y with regard to some quantitative character. We then pair first x and later y with a series of females 1, 2, 3, 4, 5, etc. From this arise two series of offspring-values which may be designated by x_1, x_2, x_3, x_4, x_5 , etc. and y_1, y_2, y_3, y_4, y_5 , etc. The average of the values $x_1 - y_1, x_2 - y_2, x_3 - y_3, x_4 - y_4, x_5 - y_5$, etc. will then afford a measure of the difference between the generative values of the two males, a measure the accuracy of which is inversely proportional to the square root of the number of females paired with the two males; the accuracy may therefore be increased by increasing that number.

ON CERTAIN FACTORS CONCERNED IN THE PRODUCTION OF EYE COLOUR IN BIRDS.

By C. J. BOND.

INTRODUCTION.

THE work of Hurst, Nettleship, and others in this country, and Davenport in America, has been concerned chiefly with eye colour in mammals, more especially in the human subject. The facts which Hurst established as to the genetic importance of the presence or absence of pigment on the anterior surface of the iris in man, seem to be applicable in the main also to the avian iris. I am unable however to find that much work has been done on eye colour in birds from the genetic standpoint, and the observations here recorded must be regarded as an attempt to carry the analysis of this problem a stage further.

I showed in 1912 (see *Nature*, Sept. 19, 1912) that in birds, not only the ciliary muscle, but the dilator and constrictor muscles of the iris are composed of fibres of the striated or voluntary kind, and that the movements of the pupil in birds are apparently subject to voluntary control. This we should expect if the iris muscles in birds are innervated by medullated nerve fibres from the cerebro-spinal system. This difference in the histological structure of the intrinsic muscles of the eye-ball in birds and other vertebrates (with the possible exception of the Reptilia) has an important bearing on the evolutionary methods by which the pigmentation of the Iris has been brought about in these different orders.

HISTOLOGICAL.

The "Bull" Eye in Birds.

By this term I mean the eye which owes its black or dark colour to the absence of pigment on the anterior surface of the iris. The delicacy and translucency of the iris tissues allow the posterior uveal pigment to shine through, and this gives rise to an appearance of blackness. The Bull eye in birds thus comes under Hurst's definition of the simplex

eye. In the Bull eye the chick character is in fact retained during adult life in the bird just as, in the blue eye in the human subject, the simplex character common to most infants (of white as opposed to negro descent) persists into adult life.

The eye of the white fantail pigeon provides a good example of the "Bull" or simplex avian eye. There is an entire absence of black, brown, or yellow anterior pigment while the connective tissue cells and the striated muscle fibres are sufficiently delicate in structure to allow the posterior hexagonal brown or black pigment to show through, and thus produce the black effect.

Careful examination of the "Bull" eye in the living bird with a lens in a good light will show a red appearance, like a ruddy glow, covering the peripheral zone of the iris. This appearance is due to a rich plexus of capillary blood vessels with thin walls in this situation, and is not due to the presence of any pigment cells.

It is important to remember that the majority of black or very dark brown irides in birds owe their dark colour to the presence of anterior iris pigment, and are not "Bull" eyes at all. Even among pigeons the "Bull" or simplex eye occurs chiefly in wholly white birds, which are genetically speaking recessive whites, though not of course albinos. Bateson and others have shown that in fowls white feather colour is of two kinds, Dominant white as in the White Leghorn, and Recessive white as in the white Rose-comb Bantam, the Silky fowl, and an extracted white obtained by crossing certain white with coloured strains. It is interesting to note that while (as we should expect) the colour of the iris in the Dominant White Leghorn is red or yellow (gravel eye) the iris colour in the Recessive white Rose-comb Bantam is not "Bull" but red or yellow.

In the Silky fowl also the black eye is not a "Bull" eye but owes its dark colour to black anterior iris pigment. There are however special points about the black eye of the Silky fowl to which I shall draw attention later.

In the Stock Dove (*Columba oenas*) a superficial observation of the eye of the adult bird would record the eye as "Bull" or simplex, i.e. deficient in anterior pigment. Microscopic examination of sections of the iris shows however that the black colour is due to the presence of numbers of branching cells packed with dark brown or black pigment granules. These cells are not only present on the surface of the iris but also among the muscle-fibres and deeper tissues in which there are also cells containing yellow pigment. Thus the black eye of the Stock

Dove is an anteriorly pigmented eye and differs from the normal type of eye colour in pigeons which is white, yellow, orange or red, producing the so-called pearl, gravel or ruby eye.

In the Rock Pigeon (*Columba livia*), which is regarded as the ancestral form, the iris colour is yellow or orange.

The Pearl Eye.

Next in grade of pigment to the "Bull" eye comes the so-called pearl eye in pigeons, and the "Daw" eye as seen in the Malay Fowl. Anterior iris pigment is absent in the pearl eye, but the iris tissues are not translucent as in the "Bull" eye. They are crowded with granules which are themselves colourless but prevent the passage of transmitted light, and when seen by reflected light give a grey white appearance to the surface of the iris.

Microscopic examination of the pearl iris shows that this opacity to transmitted light is due to the presence of these colourless, but more or less opaque, granules, which appear as clear refractile spherules of moderate size. When these are densely aggregated the interference with the passage of light may be sufficient to produce an opaque or black effect. The cells which contain these granules are scattered around the capillaries and amongst the muscle-fibres, and they do not seem to have such definite outlines as the cells which contain the ordinary yellow or brown pigment. It is indeed an open question whether these refractile bodies should be classified as pigment granules, since under dark ground illumination they appear to be of a dull white colour.

Among Fowls the eye which most nearly corresponds to the pearl eye of pigeons is the so-called "Daw" eye of the Malay Fowl.

In the "Daw" eye the iris is free from anterior yellow or brown pigment, but the connective tissue cells and the muscle fibres are loaded with granules which produce a tissue opacity and prevent the uveal pigment from shining through the iris, thus giving a grey appearance to the iris on reflected light. The pearl eye, and perhaps the daw eye, may in fact be regarded as representing the first or lowest grade of iris pigmentation, a condition of cell-opacity without cell-pigment, intermediate between the bull eye with its translucent tissue-cells and absence of pigment-granules on the one hand, and the fully pigmented eye with its connective tissue cells loaded with yellow, brown or black pigment granules on the other.

The pearl eye in pigeons seems to correspond in pigmentary gradation with the china-white or "Wall" eye in dogs, horses and pigs. We

must not lose sight of the fact however that the substitution of striated muscle-fibres in the avian iris for the plain muscle cells of the mammalian iris introduces a different histological factor in these different orders. In the mammalia there is every grade of blue eye from the deep violet or almost black shade of the newly born child or kitten to the steel blue eye of the human adult, or the bluish white iris of the blue Dutch rabbit, or the china white iris of the "wall" eyed pig.

The paleness of the blue colour or greyness of the iris depends on the granular condition or opacity of the tissue cells and the degree to which the posterior pigment is prevented from shining through. Thus, an iris composed of translucent cells gives a bull or black eye, while an iris of which the cells are slightly opaque is blue, and an iris composed of wholly opaque granular cells is china white in colour. All these irides are free from anterior pigment. The different reaction to incident light is due to structural and not pigmentary differences, and to the presence in the cells of these colourless granules.

Genetically the pearl eye in pigeons acts as a recessive to the red or gravel eye. The daw eye in fowls is also a recessive to the amber eye, and to the black eye when the blackness is due to the presence of anterior pigment.

The Yellow or "Gravel" Eye.

In pigeons the yellow eye is due to a net-work of branching cells crowded with small spherical yellow granules. These cells lie on the anterior surface of the iris: they cover the capillary blood vessels and surround the striated muscle fibres which in the pigeon are themselves free from pigment. The difference between the grey-white or pearl eye and the yellow or gravel eye in pigeons is essentially a structural difference. In the pearl eye the granules which fill these connective tissue cells are chalky white to reflected and opaque to transmitted light, whereas in the yellow eye the granules appear yellow both when viewed by reflected and transmitted light. If however the cells which contain them are closely aggregated and prevent the passage of light, then the granules appear black.

Thus the yellow eye represents the first grade in iris pigmentation. In fowls on the other hand the gravel or yellow eye presents a different problem. In some breeds the orange colour is due to the presence of large numbers of branching connective tissue cells containing yellow or yellow-brown pigment granules, which lie around the capillaries and muscle-fibres. In other breeds, of which the Dorking and

Orpington are examples, the muscle-fibre cells themselves also contain the yellow pigment granules. Bearing on this point, namely the presence of pigment granules in striated muscle cells, it is interesting to note that in some orange eyed birds the striated cells of the ciliary muscle also contain yellow pigment, and this gives in some cases a distinct yellow colour to the muscle which is quite recognisable to the naked eye. When we recall the fact that the ciliary muscle has the same embryological origin, and remains throughout individual development in close structural and functional continuity with the muscles of the iris which act on the pupil, it is a matter of interest to find that both share in the pigmentary changes concerned in the production of eye colour in some birds. It is clear that the occurrence of pigment in the voluntary muscle fibres entirely under cover of the sclerotic and unexposed to the action of light cannot have any influence in sexual selection. It must be regarded like many other pigmentary phenomena as due to an overflow of normal metabolic activity into cells which under ordinary conditions are free from such changes. The deposition of pigment granules in the iris muscle and in the ciliary muscle is chiefly found in domesticated birds. I have not, so far, come across pigmentation of the ciliary muscle in any wild bird. Such pigmentation is in fact a metabolic abnormality since it occurs in cells which in wild birds are normally free from pigmentary deposition. The effect of the deposition of a mass of pigment granules on the functional activity of a striated muscle cell is also a point of interest. It would be interesting to know whether the change affects only the older cells, it does not at any rate affect all the muscle cells even in a heavily pigmented iris. Bearing in mind that the yellow iris of the domesticated fowl is produced by the interaction of several factors: (1) the presence of yellow pigment granules in connective tissue cells, and (2) the deposition of yellow pigment granules in striated muscle cells; it becomes a point of interest to ascertain whether the yellow iris in wild birds owes its colour to the same or different factors. The owls afford perhaps the best examples of the purely yellow iris among wild birds.

In the Eagle Owl (*Bubo bubo*) the peculiar velvety bright yellow appearance of the iris is produced by a heavy coating of the front face of the iris with numbers of roundish or oval cells crowded with bright yellow spherical pigment granules. These cells are present also in Scops Owl (*Otus scops*). Probably in most, if not all of the yellow eyed owls, the yellow colour is due to the presence of these cells. The cells themselves are fairly constant in size and outline. They are free from

branching processes and gradually lose their colour when submitted to the action of 5% formalin solution.

In the Brown Owl (*Strix alues*) and the Grey Eagle Owl, and some other brown eyed species, the brown colour of the iris is due to the presence of branched pigment cells containing brown granules. The same occurs in the Eagles (*Aquilinae*), the Kites (*Milvus*) and some other birds of prey. The Egyptian Kite (*Milvus aegyptius*) and Bateleur's Eagle (*Helotarsus ecaudatus*) both have dark brown irides, and in both the pigment is contained in branch cells. In no case at present has any deposition of yellow or brown pigment granules been observed in the striated muscle-fibre cells in the iris of any wild bird.

The Brown and Black Eyes.

Amongst Birds, as amongst Mammals, quite a large number of species possess anterior iris pigment which passes through all grades of brown up to black. The black iris is associated (with some exceptions, notably the Silky Fowl) with black feather colour, and in its deeper grades with black leg colour. Histologically the brown (in its darker shades) and the black iris are produced by a well-defined layer of characteristic branching cells, which contain dark brown or black pigment, on the anterior surface of the iris. These cells intercommunicate by their branches and form a plexus of pigmented cells thickest over the capillaries in the peripheral or middle zones of the iris.

The cells are much alike in different species. The body of the cell and its processes are usually crowded with pigment granules of a rounded outline and fairly uniform size. Under favourable conditions a central nucleus can be made out more or less free from pigment. These pigment cells permeate the thickness of the iris wall for some depth and are often found encircling the striated muscle cells. They are structurally continuous with the branching pigment cells in the sclerotic and the outer surface of the choroid behind the cornea, and are quite distinct from the hexagonal cells on the posterior surface on the iris. As has been already stated, they are especially numerous and heavily pigmented in birds of dark or black plumage. They are found in nestlings belonging to the Corvidae and allied species. They are also present in the newly hatched chick in the black breeds of fowls.

Genetically the black eye with its deeply pigmented branching cells is dominant over the yellow iris and other grades of iris pigmentation thus :

The daw eyed Malay hen crossed with the Black Orpington cock

gives F_1 with black plumage (with some red in the males), and all have more or less black anterior iris pigment due to the presence of these characteristic branching cells. As the F_1 chicks develop however interesting changes take place. Among the limited number of birds reared up to the present the pullets retain the black eye in adult life, while in the cockerels the iris gradually assumes a yellow colour and at the age of 9 months or earlier, if the birds are sexually mature, the black becomes a gravel or orange eye. Thus the black iris of the hen and the orange of the cock in this cross are sex limited characters as in the case of other gallinaceous birds. In the Golden Pheasant (*Chrysolophus pictus*) the iris is brown or brownish black in the female and bright yellow in the fully developed male, and to a much less marked degree the same is true of the Mongolian Pheasant (*Phasianus mongolicus*). An interesting problem arises as to the way in which this change in iris colour is brought about in the adult male. The transition is one from an epistatic to a hypostatic character, that is from a higher to a lower grade of pigmentation. The chicks of both sexes of this Malay cross have brown black irides due to the presence of these anterior pigment-containing cells and they retain this character while sexually immature. The change to the yellow colour in the developing cockerels occurs in patches on the surface of the iris and seems to be due to the removal of the cells containing brown or black pigment and of the substitution in their place of cells containing yellow pigment granules together with (in the case of the half-bred Malay fowl) the deposition of yellow pigment granules in the striated muscle cells of the iris. The question arises as to the disappearance of these brown pigment cells. Is it the result of atrophy and absorption or of migration to deeper parts of the iris? There are reasons for thinking that both factors are concerned in the process.

As the iris assumes the yellow colour the cells which contain the black or brown pigment coincidentally lose their dendritic processes and tend to become rounder in outline. In the Herring Gull (*Larus argentatus*) the yellow iris is not fully developed in the adult male until the fourth year. A careful comparison of sections of the iris in the nestling, the young male, the young female, and adults of the two sexes of the Herring Gull shows that the black colour of the nestling's eye is due to the presence of a plexus of branched cells containing brown or black pigment on the anterior surface of the iris. The female retains more or less of this dull brown colour during adult life, but in the adult male a layer of cells containing yellow pigment replaces the layer of black

pigment cells on the front of the iris, and these latter cells are found at a deeper level just above or anterior to the uveal pigment cells. The appearances in the Herring Gull suggest a downward migration of the black branched cells and a surface migration of the yellow cells. This view that the colour change is the result of cell migration receives some confirmation in the case of young Rooks and other members of the Corvidae. The anterior surface of the iris in the nestling Rook (*Corvus frugilegus*) is dotted with a number of branching cells loaded with intensely black pigment granules. These cells are most numerous at the scleral margin of the iris and are directly continuous with a plexus of similar cells which lines the sclerotic on its choroidal surface. As the nestling grows these cells become more numerous and spread over the anterior surface of the iris, as if migrating towards the pupil from the scleral margin.

The "Black" Eye in Fowls.

If the Black Orpington (black eye) male be crossed with the Buff Orpington (gravel eye) female the F_1 chickens all show some black anterior iris pigment on a yellowish background. In the pullets the brownish black colour persists in adult life while in the cockerels the black gives place to the yellow or gravel eye. Although in this cross the yellow eye seems to be a sex limited character the sharpness of the limitation is not absolute, for a few of the cockerels show in adult life a surface layer of brownish pigment on a yellow background. In the Malay (daw eye) female crossed with the Black Orpington (black eye) male the cockerels also develop a yellow eye when sexually mature while that of the pullets remains black or dark brown, and in this cross the character seems strictly sex limited. In both crosses the deeper yellow pigmentation is due to two factors as in the yellow eye in other breeds of fowls. These factors are (a) the presence of connective tissue cells containing yellow pigment granules and (b) the deposition of yellow pigment granules in the striated muscle fibres of the iris.

The "Triplex" Eye or the Eye containing two kinds of anterior Iris pigment.

Mention has already been made of Hurst's classification of the Duplex eye as one in which anterior iris pigment of some kind either yellow, brown, or black is present. In connection with this point I wish to draw attention to a group of eyes in Fowls of which the Silky, the Croad Langshan, the Houdan and some other breeds provide examples, which might be called Triplex eyes. There are, however, some objections to

Hurst's classification of eyes as Simplex and Duplex, and it might be better to divide eyes from the colour standpoint into (a) non-anteriorly pigmented and (b) anteriorly pigmented eyes, while (b) would be further divided into singly pigmented (Hurst's duplex) and doubly pigmented or (triplex) eyes. If the eye of the Silky Fowl be examined in the fresh condition, or preferably after a few days preliminary hardening in formalin, and if the anterior surface of the iris be exposed by careful peripheral detachment from the scleral margin under water and if further the anterior layer of black pigment cells be carefully scraped away with the point of a scalpel from the front of the exposed iris, an underlying layer of reddish yellow pigment comes into view. This yellow layer can in its turn be scraped away, leaving exposed the unpigmented stroma cells and the muscular fibre cells of the deeper portions of the iris, and if these be removed the posterior uveal pigment is reached. The histology of this double layer of pigment cells of different colours in the Triplex eye is of interest.

The anterior black layer is made up of a dense network of ramifying cells crowded with dark brown or black spherical granules, uniform in size, such as we have already described as occurring in the black eyes of many species of wild birds. These cells are especially numerous around the capillaries on the anterior surface of the iris. The yellow or red-yellow layer lies beneath this and, as in the yellow eye of other breeds of fowls, is made up of two factors: (a) connective tissue cells containing large numbers of yellow pigment granules (these cells are less branched than the black cells and are situated around the capillaries in striated muscle fibres); (b) a copious deposit of yellow pigment granules in the striated muscle cells. There is a marked discontinuity and absence of grading between the yellow and black pigment layers. An object lesson is thus provided in the adult individual zygote of the definite histological deposition of one unit character, viz. black pigment over a lower grade unit character, viz. yellow pigment, and a realization in the individual of the factorial composition of the gametes into epistatic and hypostatic precursory factors.

The Genetics of the "Triplex" or doubly anteriorly pigmented eye.

Experiments have been carried out to test the behaviour of these two unit characters, black and yellow pigment, in the same eye by cross breeding. Five black-eyed Silky hens crossed with a gravel-eyed Old English Game Bantam cock gave in the F_1 generation gravel-eyed cocks and mostly black over yellow-eyed hens. In the F_2 generation some

segregation occurred with a regrouping of the black and yellow characters, with the result that both were found associated with white and game colour and with fluffy and close-feather pattern. The sex limitation also persists in the F_2 generation, the black over yellow or triplex pattern being associated with the female and the yellow or duplex pattern with the male sex. In this case a secondary male sex character, yellow eye colour, is brought about by the loss of an epistatic character, black pigment, uncovering as it were a hypostatic character, yellow pigment. The ultimate causes of the shedding of this epistatic character must probably be sought for in the metabolic processes associated with the development of sex. The metabolism which develops yellow pigment granules instead of black in connective tissue cells, and also brings about the deposition of yellow pigment granules in striated muscle cells, is no doubt associated with the functional activity of certain endocrinous glands among which we must include the primary sex glands.

The Triplex Eye or Eye with double anterior pigment in Wild Birds.

The black over yellow or triplex arrangement of eye colour in the Silky Fowl is a normal example of epistatic character, black, overlying a hypostatic character, yellow pigment. Attention has already been directed to the fact that in the adult male Herring Gull the opposite condition is found. In this case a layer of bright yellow cells covers the anterior surface of the iris and overlies a layer of black branched pigment cells. Thus yellow becomes epistatic to black in the adult male bird. As we have already seen, this result is brought about partly by the removal, by atrophy, of the black cells, and partly by the migration of these cells to a deeper layer of the iris, thus uncovering the yellow pigment cells. This arrangement also constitutes a sex limited character in other species. In the adult female Herring Gull the brown colour which is common to nestling birds of both sexes persists through life, while in the male when fully mature the iris assumes a yellow colour.

These changes in eye-colour dependent on sex and age acquire additional interest from the fact that we have here a demonstration of an actual process going on in the tissues of the zygote during ontogeny, of which the hidden counter-part has previously taken place in the factorial constitution of the germ cell at an earlier stage.

The "Ruby" Eye in Birds.

We may first deal with the Ruby eye as it occurs in pigeons and doves, because the factors concerned in the production of this iris colour are not the same in all species.

In the common Ring Dove (*Turtur communis*) the ruby colour is due to the presence of a surface layer of branching cells with yellow pigment granules, which surround the capillaries on the anterior surface of the iris.

The rich ruby eye of the Victoria Crowned Pigeon (*Goura victoriae*) is due to the same cause. In this species also, as in the Doves, the red colour is common to both sexes. In the nestling Victoria Crowned Pigeon the colour of the iris is black. At the age of three months the iris assumes a bright yellow colour, but I have not been able to ascertain the age at which the yellow gives place to the ruby colour.

In the Dragoon Pigeon and some other fancy breeds all grades of the ruby eye occur, from bright red through dull red to orange and yellow. In some birds the ruby or "red currant" colour is limited to the outer zone of the iris where blood vessels abound, while the inner zone surrounding the pupil, where there are fewer capillaries, retains the orange or yellow colour.

The Ruby eye in Doves and Pigeons loses much of its brilliant red colour after death, and, as we shall see later when dealing with the chemical aspect of the problem, the red and eventually the yellow colour are both lost when the eye is placed in a reducing agent like formalin.

The ruby colour is thus a superimposition effect due to the covering of red blood vessels with yellow pigment cells. The exact way in which the optical effect is produced is a subject for further study.

The Ruby eye in the Cayenne Lapwing (*Vanellus cayennensis*) presents a different problem. Here the red colour of the iris is due to the actual presence of rounded, oval, or slightly branched cells packed with fine granules of a reddish mauve coloured pigment. These cells are situated on and among the muscle cells and the capillaries of the iris. This is the only example at present found of a colour effect in the iris due to the presence of pigment granules which could not be included in the yellow, brown or black groups. Not only are the granules which produce the red colour in the Cayenne Lapwing's iris histologically different from the yellow pigment granules which form the surface layer in the Dove's and Pigeon's iris, but they also differ in chemical composition. In the Lapwing the ruby colour persists after death and the pigment granules retain their red colour even after a long immersion in 5% formalin.

The "Parti or Zone" coloured Iris.

In some of the Birds of Paradise, notably in Lawes' Bird of Paradise (*Parotia lawesi*), and possibly in other species, striking colour effects are

produced by a differential colouring of the iris. In Lawes' Bird an inner ring of deep iridescent blue is surrounded by an outer ring of yellow pigment. This colour effect is brought about in two ways. If a vertical section of the iris taken at right angles to the surface be examined under a low power the iris in the outer or yellow zone will be found to be four or five times thicker than in the inner or blue zone. This increased depth of tissue in the peripheral zone, containing as it does the muscular fibres and capillaries, partly accounts for the opacity of the structures and prevents the posterior pigment from shining through, hence the absence of a blue effect. In addition the anterior surface of the iris in this zone is covered with a layer of yellow pigment cells.

The inner or blue zone on the other hand is a mere thin membrane, sharply defined from the outer zone by a step down as it were in thickness on the anterior surface of the iris. The thinness and translucency of the tissues in this inner zone readily allow the black uveal pigment to shine through. The greater part of the blue effect is no doubt due to the presence of this underlying black pigment seen through translucent tissues. This however is not the whole explanation. A large portion of this thin inner zone consists of very finely fibrillated spindle cells of connective tissue type. The ends of these cells break up in hardened specimens into fibrillae which separate into wisp-like fringes. These elongated cells lie over the muscle fibre cells, and if the anterior surface of the iris in this situation be examined under a low power the parallel fibrillae bring about a diffraction grating effect. This accounts for some of the blue reflection of incident light. This can be shown by varying the angle at which the light is allowed to strike the surface of the iris. At certain angles the blue effect is produced quite independently of the posterior black pigment and can indeed be obtained after this has been removed. Thus, in the case of Lawes' Bird of Paradise a brilliant colour effect is produced by a combination of three factors: (1) Thinness and translucency of iris tissue allowing the uveal pigment to shine through. (2) The absence of anterior yellow pigment cells in the inner zone. (3) A peculiar physical conformation of the connective tissue cells in this area. A parallel fibrillation of cells acts as a diffusion grating and causes light to be reflected from the anterior surface of the iris at a certain angle as blue in colour. It would be interesting to extend this enquiry to other species of Birds of Paradise and to individual birds of both sexes at different ages.

CHEMICAL.

I regret that I am unable to bring forward any detailed account of the difference between the black, brown, yellow, ruby, and pearl pigment granules from the chemical point of view. There is one definite and constant character which serves to distinguish the yellow from the black granules and that is their different behaviour to reducing agents like formalin. The yellow pigment granules lose their colour after immersion in 5% formalin for two or three weeks. A coalescence of the small granules into larger globules of yellow lipoid-like material takes place, and these larger globules may eventually merge into larger masses. In some specimens needle crystals have formed inside these yellow oil-like globules (see Herring Gull). The black pigment granules which fill the branching cells that are so characteristic of the anterior layer in the black iris of many birds are, on the other hand, highly resistant to the action of formalin; for 5% and even stronger solutions have little or no effect in dissipating the colour. The granules do not tend to coalesce into larger globules nor do they show any signs of crystalloid change. The brown pigment granules are intermediate in their chemical reactions between the yellow and the black, and are partially affected by formalin.

THE BRINDLE COLOUR IN CATTLE IN RELATION TO RED.

BY CHR. WRIEDT,
Grenlund, Grorud, Norway.

PROF. J. WILSON has suggested that brindle is the heterozygote between black and red, or dun. From Wilson's data S. Wright has concluded that there is a special unanalyzed factor for brindle. Wilson has mentioned that dark coloured Jerseys crossed with red cattle give brindled offspring.

In Tranekjær in Denmark I have examined about 100 crossbreds from Jersey bull and the red cows of the Danish islands and none of these were brindle.

The colours in the Telemark breed in Norway are red and brindle. In this breed the segregation of black is very rare.

At the agricultural schools of Sjøve and Buskerud the agricultural teachers Lalim and Meland have under my cooperation examined the colours of parents and offspring in the herds of pure bred Telemarks. The results are given in the following table:

		Red	Brindle
Brindle × Brindle	4	24
Brindle × Red	14	21
Red × Red	12	0

In these crosses no black individuals occurred, while according to Wilson's hypothesis we should expect 7 black from the mating brindle × brindle. This, taken together with the rarity of black in the whole Telemark breed, gives a clear indication that brindle is a clear cut dominant to red. In these data no certainly homozygous brindled individual is recorded, but in a brindled bull, Tom, we have a possible homozygote. Nesheim, the former leader of the school at Sjøve, has told me that he cannot remember that Tom has given offspring of any colour other than brindle, whether he was mated with red or brindled cows.

LITERATURE CITED.

- WILSON, J. 1909. "The colours of Highland Cattle." *Sci. Proc. Roy. Dublin Soc.* N. S. Vol. XI. p. 66.
WRIGHT, S. "Colour Inheritance in Mammals, VI. Cattle." *Journ. of Heredity*, Vol. VIII. p. 521.

FURTHER EXPERIMENTAL STUDIES ON SELF-INCOMPATIBILITY IN HERMAPHRODITE PLANTS.

By A. B. STOUT.

(With Plates III and IV.)

CONTENTS.

	PAGE
Statement of the problem and its relation to the old familiar sex questions	85
Review of the more recent literature	87
Presentation of new data	93
I. Relation of vegetative vigour and maturity to variations in self-fertility and self-sterility	93
1. In <i>Verbascum phoeniceum</i>	94
2. In <i>Eschscholtzia californica</i>	96
3. In <i>Nicotiana Forgetiana</i> Hyb. Hort.	100
4. In <i>Brassica pekinensis</i>	103
5. In <i>Raphanus sativus</i>	104
6. In <i>Lythrum Salicaria</i>	104
7. In <i>Linum grandiflorum</i>	105
8. In <i>Cichorium Intybus</i>	105
II. Evidence that self-incompatibility may lead to certain cases of embryo abortion	107
III. The heredity of self-compatibility in self-fertilized lines of descent in the variety red-leaved Treviso of <i>Cichorium Intybus</i>	108
1. The I_1 generation.	109
2. The I_2 generation.	109
IV. Vegetative vigour and impotence in self-fertilized lines in the variety red-leaved Treviso of <i>Cichorium Intybus</i>	116
1. A degenerate line of descent	117
2. Families of marked vegetative vigour	118
Discussion and Conclusion	120

EXPERIMENTAL studies of the compatible and the incompatible relations of the sex organs (including germ cells) in the fertilization of hermaphrodites continue to yield evidence that these relations are highly fluctuating in the progeny of a single cross- or self-fertilized

descent. There are all grades in the degree to which both self-compatibility and cross-compatibility may appear, and even the reciprocal matings of two hermaphrodites may give opposite results.

It is clear, as it has been since the time of Darwin at least, that within the species compatibility and fertility, both self and cross, is the rule, and represents the primitive condition, and that incompatibilities are the special cases. Cross-sterility within a species is a relatively rare phenomenon, often accompanied by morphological modifications in the sex organs such as dimorphism. Self-incompatibility is more common, and has evidently a selection or hereditary value which leads to its continuation and intensification whenever it appears.

The very wide distribution of homomorphic species showing self-incompatibilities (and also cross-incompatibilities to some degree) among the families of flowering plants (Gramineae, Liliaceae, Papaveraceae, Cruciferae, Rosaceae, Pomaceae, Amygdalaceae, Solanaceae, Scrophulariaceae, Cichoriaceae, and Compositae, for example) suggests that the condition has arisen many times independently, and may be one of fundamental significance. The generally accepted view that inbreeding and continued self-fertilization are of themselves injurious is certainly favoured by the evidence that self-incompatibility is so obviously a progressive character. The application of this doctrine in these cases is to some extent subject to experimental test, for it would seem that a species whose members exhibit wide variation in self-compatibility affords opportunity to test whether continued selfing is of itself injurious.

Furthermore the intensive study of the fertility of individuals that are feebly self-compatible gives positive evidence as to how the condition of self-sterility has arisen in species originally self-fertile. The marked fluctuation of the character in practically all self-sterile species is especially illuminating on this point.

In continuing my experimental studies (1916, 1917, 1918) of physiological incompatibilities further evidence has been obtained in support of the following general conclusions.

1. In all so-called self-incompatible species investigated, with the exception of *Raphanus sativus* and the dimorphic species *Linum grandiflorum* (which, however, have not been so extensively studied), self-incompatibility and self-compatibility are highly variable in their behaviour; although the variations are generally indiscriminate, there are cases of marked correlation with conditions of age or vegetative vigour operating during the period of bloom, giving such results as "end-season" and "mid-season" self-fertility.

2. There is evidence for considering that in some cases at least embryo abortion is due to physiological incompatibility.

3. The behaviour of further pedigreed progenies of self-compatible parentage in chicory shows that repeated selection does not eliminate the extreme fluctuations though tending toward the establishment and maintenance of highly self-fertile races.

4. Continued self-fertilization in chicory has not led to a decrease in the self-compatibility that exists in any given line and has not given general evidence of decrease in vegetative vigour.

5. All the results favour the view that incompatibilities arise primarily in the ontogenetic processes of physiological differentiation of sex organs, and are not determined by either individual stuffs or line stuffs of definite hereditary value. As far as general constitution is concerned similarity in parents favours fertility. In respect to the ontogenetic processes of sex differentiation the results may be taken as evidence that successful fertilization depends fundamentally on the element of similarity.

REVIEW OF THE MORE RECENT LITERATURE BEARING ON PHYSIOLOGICAL INCOMPATIBILITY IN FERTILIZATION.

The more recent report of studies with rye (Heribert-Nilsson, 1916) favours the doctrine that self-incompatibility is a relative quality rather than that any individual, or any race of this species, is absolutely self-incompatible. Heribert-Nilsson finds, in general agreement with previous investigators, that rye is decidedly self-sterile. Highly self-fertile and feebly self-fertile individuals are however to be found in any population, and he questions whether any plant of rye is really completely self-sterile.

The offspring of self-fertile plants were grown for a study of heredity. Three families were grown to the second generation (his I_3) and two were continued into the third generation. One family which was the largest in regard to the numbers grown and tested (8 plants in the second and 10 in the third generation) maintained a rather high grade of self-fertility, and all plants were self-fertile. The parent (I_1) was judged as 0.4% self-fertile; the one plant of the I_2 was 79.8% self-fertile in field isolation; the self-fertility of the 8 plants of the I_3 ranged from 10 to 68% and that of the 10 plants of the I_4 ranged from 14.6 to 74.1%.

Certain lines of descent from self-fertile parentage did not breed true. For example one parent was 4.4% self-fertile. The self-fertilities of the four progeny were 43.1%, 1.1%, 1.6% and 0.0%. In the next generation three offspring of the plant 43.1% self-fertile were all self-fertile (15.5%, 20.0% and 11.8%), and the three offspring of the plant 1.1% self-fertile were 0.7%, 0.5% and 0.0% self-fertile.

Heribert-Nilsson, none the less, concludes that these feebly self-fertile plants are really heterozygotes. When Heribert-Nilsson states that self-fertility is a recessive character in a simple mono-hybrid relationship with self-sterility, and that self-fertility segregates as a unit in heredity and is immediately constant, he contradicts his own data. His assumption of this simple Mendelian analysis is obviously on *a priori* grounds. The variations in degree of self-fertility in evidence are so great and the number of plants grown is so small that there is certainly no positive evidence that the relative physiological conditions of the sex organs are determined by line stuffs of specific and fixed hereditary values. Obviously the true conclusion is that rye plants are more or less heterozygous as to self-fertility, which is merely another way of saying that they are fluctuatingly variable in their self-compatibility.

Heribert-Nilsson finds evidences of degeneration in self-fertilized lines, both in the quality and viability of seed, and in the vegetative vigour of the offspring, but he questions whether this is due to the immediate physiological effects of selfing, or to an increase in homozygosity. It should be noted that he gives rather meagre data for the two series of sister plants of the I_4 which were most vigorous in vegetative growth. One series of five plants is described as "kraftig" but the self-fertility of its members was evidently not determined. Of another series of nine plants of which it is stated "die Mehrzahl recht kraftig" fertility was determined for only three, and these were all self-fertile (25.8%, 28.6%, 14.6%). It is, however, stated that all seeds of the I_4 were poorly developed, but it appears that the conditions of artificial isolation (glass tubes plugged with cotton) led to vigorous growth of fungi which covered the seeds as they were developing, and this may have been the real cause of the poor viability.

Marked anomalies in the appearance of incompatibilities are seen in the fact that one species may be highly self-fertile while another, but closely related species, may be self-sterile, and hybrids between such species may or may not be self-incompatible. Cases of this sort have been studied by Detjen (1916).

Eleven cultivated varieties directly derived from the blackberry and propagated vegetatively were found to be self-compatible, while of twelve varieties descended from the dewberry ten are self-incompatible. Wild plants of the blackberry (*Rubus villosus*) were found to be self-compatible. Thus it appears that *R. villosus* is a self-compatible species while *R. trivialis* is strongly self-incompatible.

Of the varieties known to be hybrids between these two species, three (McDonald, Sorsby and Spalding) are self-incompatible and one (Rathbun) is partially self-incompatible.

Further data are needed to determine fully whether the compatibility or incompatibility of the hybrid varieties is ever complete and absolute, but it is clear that the self-incompatibility characteristic of one parent appears in various degrees in the different individuals of the F_1 . It is reported that the self-compatible plants when self-pollinated "produce apparently as good fruits as when cross-pollinated." In regard to cross-compatibility it is reported that most crosses give successful fertilization; evidently no decided cases of cross-incompatibility were found.

Detjen finds that, in addition to sterility from physiological incompatibility, certain hybrid varieties of the dewberry-blackberry cross are also more or less sterile from impotence of sex organs, as is common in inter-specific hybrids.

A most decided case of variation in sex relations has been reported by Sirks (1917) in the study of cross-incompatibilities in the hermaphrodite species *Verbascum phoeniceum*. Here the reciprocal crossing between pairs of plants gives all grades of opposite results in immediate fertility. In the extremes, both crossings may be highly and perhaps absolutely compatible, or they may be incompatible, or one may be compatible and the other incompatible. In the latter case using one plant as a male and the other as a female gives full compatibility; reversing the relation gives complete incompatibility. Furthermore there are many grades in the comparative fertility of reciprocal crossings between two plants.

This evidence agrees with that which the writer (1916) has presented for chicory, but Sirks' data are much more extensive and conclusive. It may be stated here that while Sirks has not reported on the self-compatibilities of *V. phoeniceum*, my own studies with this species, to be reported later, show that in my strains at least there appears to be no tendency for an end-season change in compatibility that might lead to

different results if reciprocal matings were made at different times during the period of bloom.

These cases of difference in the compatibility of reciprocal matings point very clearly to a source of fluctuating variability by no means sufficiently recognized, and that is the complexity of the fertilization processes as revealed by cytological study. It may well make a difference which parent furnishes the male and which the female when we realize the possibility of variation offered in the complex processes of cytoplasmic fusion, nuclear fusion, pairing of homologous chromosomes and the arrangement of the pairs with reference to each other, to the nucleus, and to the cell as a whole.

Sirks recognizes that the conditions in *V. phoeniceum* indicate that "auto-incompatibilité" is a phenomenon of physiological sex differentiation which cannot be ascribed to fixed genotypic constitution nor to the inheritance of specific line stuffs. He suggests that the poor growth of pollen tubes very generally observed in cases of incompatibility may involve osmotropism.

Evidence that self-sterility is somewhat exclusive of, and more specific than, cross-sterility is given by Sutton (1918). The evidence of a wide range of variation in self-compatibility among the various cultivated (propagated asexually) varieties of plum, of cherry and of apple has been confirmed by studies of varieties commonly grown in England. Self-fertile, partially self-fertile and self-sterile varieties are reported in each of these quite as have been found by other investigators. Sutton finds, however, no evidence of cross-incompatibility between varieties except in crosses between the Jefferson variety and the Coe group of varieties of plums. She concludes that otherwise inter-varietal cross-fertility under field conditions depends solely on the production of plenty of pollen and on simultaneous blooming. The varieties of the cherry reported cross-incompatible by Gardner (1913) were not studied by Sutton.

Sutton's data show clearly that a distinction is to be made between fruitfulness involving only parthenocarpy, and fruitfulness with and dependent upon seed reproduction. In the case of the navel orange the size and quality of the carpels are quite independent of any process of fertilization, or even of parthenogenetic production of seed; the pistils however contain normal ovules, and when pollination occurs fertilization results and seeds are formed even in the accessory carpels (Shamel, 1918). Sutton finds in plums and cherries that well formed fruits

usually contain well developed seeds; there is close correlation between fruitfulness and seed production, as perhaps is the rule in the great majority of seed plants. In the apple she finds that seedless fruits are frequent and that in some varieties seedlessness does not involve any decrease in the size of the fruit. In some varieties, however, there appears to be a decided inter-relation between fruit-development and seed-development as Ewert (1909) and Kraus (1915) have particularly pointed out. The relation between the conditions which lead to parthenocarpy and those which lead to bulbil formation for example is by no means clear. Evidently there are various types of parthenocarpy; in some cases it is apparently a purely vegetative phenomenon, in other cases pollination seems necessary for its initiation.

The conditions in the fruit-sterile (to self-pollination) and the seed-sterile (seedless) varieties of apple also raise some question as to the stage at which fertilization fails in these cases. Kraus is of the opinion that in self-sterile varieties generally the union of the proper nuclei within the embryo sac is apparently normal (Kraus, 1915, p. 554). If this be true then the incompatibilities come to their expression after fertilization, perhaps as embryo abortion. Ewert (1909) however holds that the more or less rudimentary seeds in the so-called seedless, and in the feebly self-fertile varieties, are largely, if not entirely, due to parthenogenesis. Sutton states that "the stage at which fertilization fails probably differs in various forms."

Recent papers by East and Park (1917), and by East (1918), extend considerably our knowledge of self- and cross-incompatibilities in certain species of *Nicotiana* and their hybrids, and decidedly modify previous statements of fact and theory for these species.

It now appears that *N. Forgetiana*, *N. alata*, *N. glutinosa*, *N. angustifolia*, and various of their hybrid offspring may be self-compatible to some degree and that cross-incompatibility may also be strongly in evidence. The variability of the relations, both self and cross, is hence much greater than was previously reported by East (1915), and in this general condition these species of *Nicotiana* are apparently not fundamentally different from other self-incompatible species.

East and Park also present very interesting evidence that sex compatibilities may show cyclic changes, becoming stronger with the full maturity of plants. They find a decided tendency for incompatibility, both self and cross, to appear during the period of vigorous bloom, and then to disappear near the end of the blooming period.

This indicates most clearly, as I have pointed out above, that incompatibilities, and especially self-incompatibilities, are acquired, and may, under special conditions, give way to the original primitive condition of full self-fertility. The whole set of conditions favours the conception that compatibility depends on similarity of gametes. Acquired differences which result in self-incompatibility have marked selective value only to the advantages in variability resulting from limiting sexual reproduction to crossing between individuals or races.

Further evidence of variability in the relations of incompatibility is seen in the fact that self-fertility is more pronounced in *N. alata* than it is in *N. Forgetiana*. It is interesting to note that East and Park consider that these conditions and differences can be so fully disregarded in judging heredity that each species can be called fully self-sterile, and be described as homozygous for a single unit factor (or possibly multiple factors) solely concerned with the hereditary transmission of self-incompatibility.

East and Park have made the most extensive studies of reciprocal crosses that have thus far been reported. Their facts show a considerable variation in the reciprocal relations of two individuals, but they believe that this is solely due to experimental error and to differences in maturity of the individuals, and that reciprocal matings should give the same results provided end-season conditions are not involved. They decide, therefore, that the condition of compatibility or incompatibility between sex organs (including the gametophytic generation) is determined for a plant as a whole rather than for sex organs as such. This view is decidedly at variance with the results which Sirks, and also the writer have found, as noted above.

East and Park consider that inbreeding or breeding from self-fertile plants increases the amount of cross-incompatibility; the marked or very general cross-incompatibility of a progeny being ascribed to increased homozygosity. This assumption seems to have some evidence in its support, but it has by no means been rigorously tested and adequately proven.

The point of view of East and Park is that incompatibilities, both self and cross, are not fundamentally phenomena of sex differentiation, but are properties of plants as wholes predetermined by line-stuffs. The emphasis is placed on a Mendelian description in terms of hereditary units. They recognize that characters and factors representing them are very generally variable, but prefer to regard the marked variations in self-fertility as a "pseudo" fertility of no genetic significance and to

assign the decided variation in the grouping of cross-incompatibilities to variations in linkage relations.

The available data on cross-incompatibilities in different species indicate marked variation in the group relations. East and Park report that the members of a seed progeny fall into classes which exhibit intra-class sterility but complete inter-class fertility. Thus if any two plants of a progeny are cross-sterile they will behave the same to a third plant. This rule does not agree with the relations reported by Correns (1912) for *Cardamine pratensis*, nor with the still different relations which Sirks reports for *Verbascum phoeniceum*.

East and Park give conclusive evidence that there is wide variation in the number of groups, and the number of individuals in the various groups within a seed progeny. For example, in one progeny of 53 plants there were three well defined groups of 22, 16 and 12 individuals; in another there were five groups of 8, 3, 4, 3 and 2 plants; another progeny of only 18 plants fell into six classes (4, 5, 3, 2, 2 and 2); another was composed of six classes of 4, 5, 7, 5, 3 and 3 plants; another showed marked inequality of four classes with 34, 11, 4 and 2 individuals per class. East and Park assume that these irregularities are due to variability in linkage relations. Their further assumption that the pollen grains of a plant all operate alike does not hold for many feebly compatible self- and cross-fertilizations that are operating periodically or indiscriminately. The variations in cross- and group-relations are quite in harmony with the wide variations that appear in the sex relations of the organs produced by individual plants.

PRESENTATION OF NEW DATA.

I. *Relation of vegetative vigour and maturity to variations in self-fertility and self-sterility.*

End-season fertility is one of the clearest evidences of the fluctuating nature of the relations of the sex organs in plants that are feebly self-incompatible. It indicates, as does also mid-season self-fertility, the cyclic nature of life processes, and supports the doctrine that sexuality is itself fundamentally a function of maturity.

In the experiments here reported controlled self-pollinations at frequent dates throughout the entire period of bloom were made. The evidence is clear that end-season fertility is comparatively rare, and is not a condition commonly operating in and characteristic of self-

incompatible species. In the species in which it is found it occurs together with variations that operate from the first day of bloom.

Verbascum phoeniceum.

The habit of growth in this species is especially favourable for the study in question. Flowering begins when the plants are still in vigorous vegetative condition. The flowers are borne on loose racemes which make most of their growth after the first and lowermost flowers bloom. The rosette leaves begin to die during the flowering period, are usually dead at the end of the period of bloom, and the stems and their leaves, and often the whole plant, die at the maturity of fruit. The most terminal flowers usually fail to develop, obviously because of waning vigour. Flowering begins when the plant is in vigorous vegetative condition and continues during the gradual decline in vigour and the approach of death.

The entire main raceme can be enclosed in a semi-transparent "glassine" bag which can be shifted as the raceme elongates. Lateral branches which bloom somewhat later than the main stem may be treated likewise. In testing the plants pollinations were made at intervals of from 2 to 5 days, as conditions admitted, throughout the entire period of bloom. All pollinations were made by hand during the forenoon when corollas are not wilted and the freshly opened stamens contain much pollen. Flowers thus treated were properly tagged, and record made in a card file which was carried in the field. Forceps used in the manipulations were dipped in alcohol and the hands were washed in water after each plant was worked. Under such treatment plants highly self-fertile set fruit and seed throughout the entire period of bloom, and compatible crosses were likewise successful.

A series of 69 sister plants was grown in 1918 from seed of a cross between two self-sterile plants. These parent plants had shown themselves self-sterile throughout the first half of their period of bloom in the case of about 50 flowers self-pollinated under control. The seed was sown in January 1918 and the seedlings were grown in pots until May when planted in rows in the garden. Under this treatment flowering began in June. The periods of bloom ranged from 16 to 39 days. The number of flowers hand-pollinated per plant ranged from 45 to 244, and the total number of flowers hand-pollinated was 7703.

Two plants were highly self-compatible, 9 were feebly self-compatible and 58 were completely self-incompatible. The two first mentioned

produced fine capsules from nearly every flower that was self-pollinated by hand (numbers were 58 and 183). In feebly or partially self-fertile plants, as a rule, a small proportion of flowers produced pods, and these were small and contained relatively few seeds; seven of these were feebly self-fertile from the first few days of bloom to the end of the blooming period.

For one plant, judged as partially self-fertile, pollinations were made on 14 different dates for a total of 244 flowers of the main and three lateral racemes. A total of 49 pods were produced and these were scattered over the racemes from the first to the very last of the flowers that were pollinated; many pods had no seed and the highest number for any pod was 18.

The following is the record for one (No. 8) of these self-fertile plants. The dates of pollination are given in *italic*, the number of self-pollinations that failed is indicated by *roman*, and the number of flowers that produced pods is given in **bold face**. The main raceme began bloom *June 6*, 2 flowers not pollinated; *8th*, 4 + **1**; *10th*, 9; *12th*, 4 + **2**; *13th*, 4 + **3**; *17th*, 12; *19th*, 8; *21st*, 4; *25th*, 3; *28th*, 3 which were the last on the main stem: for a large lateral branch from base of plant; began bloom *June 24th*; *25th*, 7 + **3**; *28th*, 11; *July 3rd*, 9 + **1**; *9th*, 10; *13th*, 6. The ninth plant classed as self-fertile produced 13 pods out of 26 flowers pollinated on first four dates, but gave not a pod in the 76 flowers self-pollinated on later dates.

Not a single pod was produced in the controlled self-pollinations by any of the 58 plants judged to be completely self-incompatible. As many as 288 flowers for a single plant were selfed and special effort was made to continue pollinations in one or more lateral branches as well as in the main branch until the last flower bloomed. One of these plants had a second period of bloom. The main stem and its immediate laterals bloomed from June 13 till Aug. 18. Later a branch arose from among the rosette leaves which bloomed from Sept. 14 until Oct. 4. The flowers on this branch were all self-sterile.

Every plant of this series was highly productive in pods when the flowers were subject to free open cross-pollination by insects. Numerous tests of the self-fertile plants by emasculation of flowers showed that parthenocarpic fruits and apogamous seeds were not formed.

Another series of 40 sister plants was grown from seed of a plant that was highly self-fertile from the first date of bloom.

Twenty-four of these plants were almost completely impotent as males but fully potent as females. The stamens were contabescent, not a

96 *Self-Incompatibility in Hermaphrodite Plants*

single anther sac was observed to dehisce, and repeated examinations of the contents showed that at least 95 % of the pollen was shrivelled and empty. On three other plants, a few anthers were observed to dehisce partially, but in these the pollen was likewise largely impotent. All these plants produced corollas that opened normally, and every one produced large capsules with seed to free open cross-pollination. These plants exhibited a one-sided impotence identical with that observed in various species, such as in certain so-called self-sterile varieties of cultivated grapes and of the navel orange.

Twelve plants were fully potent both as males and females. They bloomed from 16 to 28 days, and controlled self-pollinations were made for from 20 to 64 flowers per plant. Five were completely self-incompatible, two were partially self-fertile, and five were highly self-compatible. The latter gave fine large pods in nearly every flower from the first to the very last to bloom. Of the partially self-compatible plants, one produced pods only on first two dates of selfing, and the others produced numerous but small pods indiscriminately throughout the period of bloom.

Summary. There was no evidence of "end-season" self-fertility in any of these plants. Plants were strongly or feebly self-fertile from the first flower that opened, and all plants completely self-incompatible during the first part of the blooming period remained thus to the end. In some cases of feeble self-fertility the self-fertility appeared to be confined to the early and mid-season period of bloom.

Eschscholtzia californica.

For the seed of this species the writer is indebted to Professor W. T. Horne, who collected it from individual plants growing wild on or near the campus of the University of California. Seeds of each of 5 plants were sown separately during March, 1918. All but three of the plants were grown in pots in a greenhouse. Flowers were enclosed in glassine bags and allowed to make autonomous self-pollination, a procedure that supplies an abundance of pollen to stigmas at a time when they are receptive to compatible cross-pollinations. The dehiscence of the stamens of individual flowers continues for several days, during which time the stigmatic branches of the pistil elongate, coil about among the stamens and become covered with pollen. Pollen and stigmas in this stage used in crossing most often give a rapid development of fruit and seeds.

Series 1. Twenty-nine sister plants bloomed from 15 to 58 days. From 5 to 15 flowers per plant, and a total of 236 flowers, were allowed to self-pollinate under bags. Special effort was made to include the last flowers that bloomed.

Seventeen plants were completely self-incompatible and did not even produce small pods. Four plants produced small pods in nearly all flowers selfed but all the pods were empty. Eight plants produced pods for nearly all flowers selfed, and some seeds were present. The total number of seeds per plant for these was as follows: 14, 10, 5, 3, 2, 2, 1, and 1. One of these gave 2 and 12 seeds in the second and third flower that opened, while the 8 selfed on later dates gave only empty pods. Another plant gave 1 and 4 seeds in the first and third flowers to open. Another gave 3 seeds for 1 flower, the next to the last to open. The others produced seed as noted in single pods for flowers that opened near the mid-season of bloom.

A plant of this series was planted in my home garden and allowed to bloom in the open. Under such treatment many more flowers are produced than when grown in pots. The plant bloomed from June 13 until Aug. 19. As many as 245 flowers were tagged for special observation. In the majority of cases no semblance of pods developed; in a small proportion, rudimentary pods about 2 cm. in length developed. The third flower to bloom produced a pod 3 cm. in length with 3 seeds; other pods from 2.5 to 3 cm. in length were produced for flowers opening on the following dates and contained seed as indicated: *June 30th*, 3 seeds; *July 1st*, 6 seeds; *2nd*, 5 seeds; *4th*, 2 seeds; *6th*, 1 seed; *8th*, 3 seeds; *12th*, 3 seeds. Many flowers opening on the same and on intervening dates produced no pods and seeds, and 143 tagged flowers selfed on 21 dates later than July 12, all failed to produce seed. This plant was repeatedly crossed with a plant of another series and proved to be highly productive of seed, giving from 70 to 101 seeds in capsules that were often 10 cm. in length.

Series 2. Forty-one plants descended from a single seed parent were grown in pots. They bloomed for periods of from 12 to 63 days. From 5 to 18 flowers per plant were self-pollinated under bags. The total of all flowers thus treated was 337. Fourteen plants were completely self-sterile and produced no pods; 15 produced small pods in nearly every flower but these contained no seeds or only shrivelled rudiments of seeds; 11 plants produced small sized pods (2 to 6 cm. in length) with only a few seeds; and 1 plant was highly self-fertile. The results for some of the 11 plants feebly self-fertile may be given.

98 *Self-Incompatibility in Hermaphrodite Plants*

One plant bloomed for 51 days; 18 flowers selfed produced 18 pods, none over 5 cm. long; 6 pods contained seeds as follows: 1, 2, 3, 2, 1, and 3, all in flowers that opened during first 28 days of bloom; no seeds were produced in 7 flowers self-pollinated during the last 23 days of bloom.

Another plant bloomed for 58 days, 8 flowers were selfed, all produced pods with seeds in 5 pods as follows: 1, 1, 3, 7, and 2, and in this case the first flower to open produced 1 seed and the very last 2 seeds.

One plant produced 2 seeds in each of the first 2 flowers to open but none in the 7 flowers tested later.

One plant gave 9 and 6 seeds in 2 pods from flowers that opened during the mid-period of bloom and none in flowers pollinated earlier or later.

The other seven of the partially self-fertile plants produced capsules and seeds as follows: 13 capsules, 1 seed; 11 capsules, two with 1 seed each; 6 capsules, one with 2 seeds; 13 capsules, 1 seed; 8 capsules, 1 seed; 8 capsules, 1 and 2 seeds; 5 capsules, 1 seed. In all of these the capsules bearing seed were from flowers that opened before the mid-date of bloom.

The single plant that was classed as highly self-fertile bloomed for 18 days and had only 6 flowers; three of these self-pollinated, gave fine pods bearing 26, (seed lost), and 15 seeds each. The first flower to open gave 26 seeds.

One plant of this series was grown in isolation in the field. It grew vigorously, made a spread of nearly 8 feet in diameter, began bloom on June 21st, and was still blooming on Nov. 6th when freezing temperatures occurred. During the latter part of July and the first week of August as many as 100 flowers opened each day. Insect visitors were abundant during most of the flowering period and carried much pollen from flower to flower, besides the autonomous fall of pollen to stigmas. The plant was examined at intervals of about every third day and during the earlier and later portions of the blooming period all flowers opening on those dates were tagged. During the height of bloom many of the flowers (up to 35 per day) were tagged. A total of 928 flowers were tagged and examined later, and at least as many more opened. Not a single ovary enlarged to a size of more than 1.5 cm. in length. The plant was completely self-sterile producing neither seeds nor empty pods. On four widely separated dates the plant was crossed with a plant of *Series 1*, and every pistil produced a fine large pod with many seeds.

Series 3. There were 43 plants in this series all of which were grown in pots. Periods of bloom varied from 28 to 69 days. The number of flowers self-pollinated per plant ranged from 6 to 25 and the total was 474. Thirty-six plants were completely self-sterile and did not produce pods. For 5 plants, empty pods of small size developed for flowers selfed. One plant gave a single pod with 6 seeds out of 18 flowers self-pollinated, a result that may be attributed to experimental error. Large fine pods with numerous seeds were frequently and very generally produced from flowers that bloomed in the open and were subject to cross-pollination by insects.

Series 4. The 46 sister plants of this series grown in pots bloomed for periods of 11 to 70 days. From 6 to 18 flowers per plant were selfed under bags, and the total for all such flowers was 513.

Thirty-two plants were completely self-sterile to the extent that pods were not formed. For 11 plants the pistils of flowers selfed enlarged somewhat and occasionally one was so much as 4 cm. in length, but all were either entirely empty or contained only shrivelled seeds.

Three plants produced seeds as well as pods. For 1 plant, only 1 pod had seed (the number was four) and this was of a flower that opened 24 days after first flower, and 20 days before last flower of the period of blooming. On another plant, 3 seeds from the first flower to open, and 3 seeds from a flower that opened near mid-season were all that developed for the 10 flowers selfed. A third plant produced 2 seeds for a flower that opened at mid-bloom while 7 selfed on later dates gave no seed.

One plant was grown in isolation in the field. It bloomed for 40 days, and 97 flowers in all were tagged for special observation. In these as in all other flowers no pods developed.

Series 5. Thirty-nine plants of this series were grown in pots. The periods of bloom ranged from 13 to 62 days. The flowers selfed under bags ranged from 4 to 13 per plant, and the total for all such flowers was 263. Twenty-nine plants were completely self-sterile forming no pods; 8 produced empty pods of small size; 2 plants produced seed as follows: for 1 plant 12 flowers were selfed and the first flower to open gave 3 seeds, all others produced empty pods: for another plant of 9 flowers that opened, 4 were selfed and these gave 6, 8, 7 and 8 seeds respectively.

Summary. In these five series the greater number of plants were completely self-sterile throughout the entire period of bloom. Self-compatible plants were in larger proportion in some series than in

100 *Self-Incompatibility in Hermaphrodite Plants*

others. With only one exception the self-compatibility was feeble. It is clear that self-compatibility exhibited no tendency to develop as an "end season" fertility.

A most striking type of seed sterility was seen in the considerable number of plants that produced pods which were empty. In the feebly self-fertile plants, as a rule, the few good seeds that were developed were located in the upper end of the capsules. The considerable number of poor seed present both in pods containing few seeds, and in empty pods, suggests that incompatibility may operate after fertilization, producing embryo abortion. The results here obtained also suggest that some seedless pods may develop as a direct result of the stimulus of pollen-tube growth, and more obviously that the size of pods bears a relation to the number of ovules fertilized.

Nicotiana Forgetiana Hyb. Hort.

Series 1. In 1917, the writer, assisted by Miss Helene Boas (now Mrs. Cecil Yampolsky), tested the self-compatibility of 58 plants of *N. Forgetiana* Hyb. Hort. Controlled self-pollinations were made by hand on successive dates using the same method employed for *Verbascum phoeniceum*, except that bags were shifted from branch to branch. In all cases the pollinations were begun during the first days of bloom.

On the basis of their self-compatibility and incompatibility these plants may be grouped in three classes.

(1) Eleven plants were highly self-fertile from the first day of controlled self-pollination. There were only 3 flowers out of 95 selfed that failed to produce pods. The capsules were large and well filled with seeds. As soon as it was found that a plant was self-fertile no further controlled pollinations were made.

(2) Eight plants were feebly self-compatible. Capsules were produced in relatively few of the selfed flowers, and these were small and contained few seeds. For 6 plants some pods developed in flowers of the first date of selfing; in the other 2 plants pods were formed from flowers of the second date of selfing. All these plants, however, were partially self-compatible during the first days of bloom.

(3) Thirty-nine plants were completely self-incompatible to all controlled selfings made, which were extended to well past the climax of bloom in all cases and for 10 plants the selfings were continued to the last date of bloom.

The plants of this series were grown from commercial seed and there was considerable variation in the colour of the flowers. Several con-

formed closely to the original description (Hemsley, 1905) of *N. Forgetiana*, but these were also plants whose flowers were of lighter and of darker shades of red, and there were green-flowered and white-flowered plants.

It may be noted here that no pure stock of this species was ever kept in culture from the original and only importation of seed obtained from a wild source. In reply to inquiry, the author learns the following by letter from the firm of Sanders of St Albans, England, who made the importation: "We never sold any of the original stock. We simply kept them for hybridizing purposes, afterwards selling the entire lot together with the seed. We had previously secured a batch of really fine new crosses with *N. affinis*, etc. resulting in a lovely set. When these plants were in full bloom in all the shades of rose, purple, white, pink and crimson the effect was magnificent. We did not continue with the growing of the *Nicotianas* but sold our stock. We regret very much having lost this remarkable species."

Series 2. A self-fertile plant of the 1917 crop which appeared to be identical with the original description of *N. Forgetiana* was the parent of 24 plants grown under field conditions. Guarded self-pollinations were made by hand at frequent dates from the first to the very last day of bloom.

Eleven plants were entirely self-incompatible. They bloomed for periods of from 22 to 65 days, and the total of all flowers selfed was 377.

One plant was judged as fully self-compatible. It produced fine pods in all flowers selfed on the first, second and fifth days of bloom. No controlled selfings were made thereafter.

The other 12 plants were partially or feebly self-compatible. The complete data for these would occupy much space, but as the results have a very special bearing on the variations in self-fertility they can be summarized, at least for several plants which are typical for the results.

One plant began blooming on July 27 and was still blooming on Nov. 6 when there was a heavy frost. A total of 80 flowers was selfed on twenty different dates. On the third day of bloom the fourth flower to open produced a pod with seeds; 2 pods developed from the 2 flowers that opened on the fourth day; one of 2 flowers selfed on the twelfth day developed a pod; 3 selfed on the seventeenth day gave pods; on Sept. 4th, 6 out of 10 flowers selfed gave pods, but on ten dates immediately preceding, self-pollinations of 27 flowers all failed. From Sept. 4 until the end of the season about half of the flowers selfed produced pods and seeds. In respect to the proportion of flowers which

102 *Self-Incompatibility in Hermaphrodite Plants*

produced pods, the self-compatibility of this plant appeared to increase toward the end of the period of bloom. The plant was, however, somewhat self-compatible from the first.

Another plant bloomed from Aug. 29 until Oct. 24. Two small pods were obtained from two flowers selfed on the tenth day of bloom while 16 flowers selfed on five earlier dates entirely failed; 51 flowers selfed on seventeen later dates, and including several of the last to bloom, all failed.

For another plant, of 38 flowers selfed on eleven different dates, there were 10 pods of scattering dates from the first day of bloom.

A plant that bloomed for 80 days was tested in 45 flowers on eighteen dates. Only two small pods developed; one on the eighth, and one on the twenty-sixth day of bloom.

Another plant gave 10 pods at scattering dates from the fifth day of bloom to the last date, 55 days later.

A plant which bloomed for 63 days was tested in 51 flowers; two small pods were produced, one on second day of bloom and one on the thirtieth. Twenty-two flowers selfed on later dates failed.

The complete record for one plant may be here given with dates of pollinations, failures indicated by 0, and capsules by P. *Aug. 6th*, first flower, 0; *9th*, 0, 0; *12th*, 0; *14th*, 0, 0; *16th*, 0, 0, 0; *19th*, 0, 0; *21st*, 0, 0, P; *24th*, 0, 0; *Sept. 4th*, 0, 0; *6th*, 0, 0, 0, 0, 0, 0, 0, 0, P; *9th*, 0, 0, 0, 0, P; *11th*, 0, 0, P, P, P; *14th*, 0, 0, 0, 0, 0; *16th*, 0, P, P; *21st*, 0, 0; *24th*, 0, 0, 0, 0; *28th*, 0, 0, 0; *30th*, 0 and the last flower. This plant was feebly self-fertile but its self-compatibility was mid-seasonal rather than end-seasonal.

Six sister plants of this series were grown to maturity in pots in a greenhouse, a treatment which greatly reduces the number of branches and flowers. These plants bloomed for periods of 9 to 51 days. Four were completely self-incompatible. Two were feebly self-compatible. For one of these the record is as follows: *Aug. 10th*, began bloom, 0, 0; *14th*, 0, 0, 0; *16th*, 0, 0; *19th*, 0, 0; *22nd*, 0, 0, 0; *Sept. 3rd*, 0, 0, 0, P; *9th*, 0, 0, P; *11th*, 0, 0, P; *16th*, 0, P. This plant exhibited a good case of end-season self-fertility. Another plant bloomed for only eighteen days and produced but 10 flowers, 6 of which were selfed with the following results: *Sept. 3rd*, 0; *9th*, 0; *11th*, P; *16th*, P, P; *20th*, 0.

All the self-sterile plants produced fine pods for the greater number of flowers which bloomed in the open subject to cross-pollinations by insects and humming birds. Numerous tests for parthenocarp and apogamy were made in self-fertile plants but in no case did pods form.

All plants of this series were remarkably uniform in general habit of growth and in flower colour. In all respects the plants seemed to conform closely to the characteristics of the original plants of *N. Forgetiana*.

Series 3. These plants were grown from selfed seed of a green-flowered plant which was highly self-compatible from the first day of bloom. Of the 13 plants grown in the field, 12 were highly self-compatible in every flower tested during the first five days of bloom; no later tests were made. One plant was completely self-incompatible; it bloomed for 58 days, was tested on twenty dates with a total of 64 flowers including some of the very last to flower.

Of 4 sister plants grown in the greenhouse, 3 were fully self-compatible and 1 was feebly self-compatible from the first day of bloom.

This series was very uniform in general habit of growth and in flower colour. The corollas were greenish white with the inner face becoming white with age.

Summary. The results show conclusively that there were plants fully self-compatible or completely self-incompatible throughout the entire period of bloom. The partially self-compatible plants were rather irregular in production of pods to selfing. Many were partially self-fertile from the first date of bloom and exhibited no decided change in this relation thereafter; in others the self-compatibility was most pronounced or was confined to the earlier part of the period of bloom, to the season of mid-bloom, or to the last part of the blooming period. The cases of decided end-season self-fertility were few, and constituted a small proportion of the partially self-compatible plants.

Brassica pekinensis.

Experiments with this species have given results which show that a few plants are highly self-fertile, but that the greater number are self-incompatible. At least some of the self-compatible plants exhibit several well defined stages or types of sterility in the succession of bloom: (1) flowers blast or fail to develop; (2) flower parts develop and open normally but abscission of pistil soon follows after self-pollination, or the pistils remain attached for a longer time but fail to develop into pods; (3) a period of fruit and seed production; (4) a period same as (2); and (5) failure of flowers to open normally or even their blasting as in (1). In self-incompatible plants the period of fruit and seed production to selfing is omitted. In certain plants stage (1) is omitted. In one self-fertile plant stage (5) was omitted in the main branches. The lateral

104 *Self-Incompatibility in Hermaphrodite Plants*

branches which bloom later than the main ones usually omit certain of the earlier stages exhibited by the latter. The evidence seems conclusive that when compatible cross-pollination is made there is production of fruit and seed in any stage except (1) and (5). The highest degree of self-compatibility appears to develop during the mid-season of bloom.

During the latter part of the period of bloom (designated above as period 4) there is usually a poor development of pods and these may contain only partly formed seeds in which embryo abortion has taken place. This is evidently due to the rapid decline and death of the entire plant.

Raphanus sativus.

It can here be reported that some cultivated varieties of this species are decidedly self-incompatible. Nearly 50 plants of three varieties grown to maturity in a greenhouse have completely failed to produce pods to self-pollination. Of plants tested thus far only one has shown signs of self-fertility. It was grown in the field, bloomed from June 22 to July 13, and produced 223 flowers many of which were selfed by hand. Six small pods were produced, each containing from 1 to 2 seeds. All these were for flowers which opened during the mid-season of bloom. Further studies with varieties of the radish and also of the Chinese cabbage are in progress.

Lythrum Salicaria.

The marked sterility of this trimorphic species to self, to intra-form, and to inter-form illegitimate pollinations was noted by Darwin (1865). His experiments revealed, however, that self-fertility is somewhat in evidence especially in the mid-styled plants.

My own experiments with this species have thus far been confined chiefly to the growing of plants in isolation for the observation of seed production when subjected to free self-pollination by insects. The results thus far obtained show (1) that short-styled plants set very few pods; (2) that long-styled plants are feebly self-fertile and that (3) mid-styled plants are often decidedly self-fertile. One mid-styled plant grown under constant observation in my own garden has shown a decided increase in fertility at the end of the period of bloom. All other plants tested have shown quite a uniform degree of self-compatibility throughout the entire period of bloom.

End-season self-fertility in this species has to date been observed in only one plant. This condition seems to develop as an individual

variation and not as a phenomenon characteristic of self-incompatible plants.

Linum grandiflorum.

Numerous tests for self-fertility have been made especially at the end of the period of bloom for both long- and short-styled plants of this species. Plants that made a vigorous growth in the field have been cut back, replanted in pots and grown during the winter in a greenhouse. Such plants bloomed profusely in isolation and hundreds of self-pollinations were made by hand. All self-pollinations have failed completely. The species appears to be completely self-incompatible. All intra-form crosses which were made failed, but there was always marked fertility in inter-form crosses. The relations in crosses were however not extensively tested.

Cichorium Intybus.

It should perhaps be stated here that the evidence already reported for wide variation in self-compatibility in this species does not involve a mingling of data collected at early and late periods of bloom, nor has there been any comparison between such data. An examination of the records for dates and periods of flowering shows that in no case have controlled pollinations been begun later than the tenth day after the first flower head opened, and that for 95 % of all plants studied pollinations were begun during the first three days of bloom and continued over an interval which seldom extended to the mid-date of bloom.

In the more robust races, and especially in the variety red-leaved Treviso, the period of blooming often continued until plants were killed by heavy frosts early in November. Toward the end of the period of bloom relatively few flower heads open, and these are rather scattered. During the latter part of September and in October many flower heads open irregularly and poorly, and when plants are killed by frost the seed in many heads is immature. I have therefore never made any pollinations of this species later than the 6th of September. It has been most convenient, and it has afforded a most uniform treatment as well, to begin pollinations within the first three days of bloom and to continue until about the tenth day. Especially for the plants of the variety red-leaved Treviso this covered but a small part of the period of bloom.

Controlled self-pollinations have been continued for a longer period than was the rule, in the case of certain plants found to be self-compatible and from which considerable seed for planting was desired. Seed pro-

106 *Self-Incompatibility in Hermaphrodite Plants*

duction has been somewhat irregular but there has been no case of decided increase in self-fertility as the plant progressed in bloom. Cases showing the opposite condition have been found, but this was not known in time to make tests to determine if *all* seed production was not also decreased.

Efforts have been made to test for end-season fertility, especially in a line of red-leaved Treviso which has a somewhat shortened period of bloom. In 1918, 10 plants, the first to flower in each of two series, were thus studied. The plants that were completely self-incompatible during the first ten days of bloom, in tests of about 30 flower heads, remained completely self-sterile throughout. The only decided variation in fertility observed in self-fertile plants was in the direction of a decrease. Data may be given in detail for 4 plants which illustrate the results obtained.

Plant *R 12-11-59, no. 53*. A plant feebly self-compatible from the first day of blooming and judged to be 4% self-compatible. Bloomed from July 11 to Aug. 21. The dates of pollinations and number of seed per head were:—*July 12th*, 0, 0, 0, 2, 4, 7; *16th*, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 7, 8; *22nd*, 0, 0, 0, 0, 0, 0, 0, 0, 0, 2; *25th*, 0, 0, 0, 0, 9; *29th*, 0, 0, 0, 0, 1; *Aug. 2nd*, 0, 0, 0; *15th*, 0, 0, 0, 0, 2, 2. From Aug. 5th until the 21st few and scattered flower heads were produced and the branches were dying and becoming dry and brittle. The heads tagged for selfings were well distributed over the plant.

Plant *R 12-11-59, no. 2*. Bloomed from July 1 until Aug. 20. Record as follows:—*July 2nd*, 0, 5, 11; *5th*, 0, 3, 6; *8th*, 0, 0, 0, 0, 1, 3, 8; *12th*, 0, 0, 0, 0, 0, 2, 4, 9; *16th*, 0, 0, 0, 0, 0, 0, 0, 0; *Aug. 8th*, 0, 0, 0, 0, 0; *12th*, 0, 0, 0, 0, 1. These results seem to indicate that the plant was feebly self-compatible at first but less so later.

Plant *R 12-11-59, no. 6*. Bloomed from July 12 until Aug. 23. A completely self-incompatible plant. Dates and number of heads given:—*July 12th*, 2; *15th*, 15; *18th*, 5; *22nd*, 12; *25th*, 6; *29th*, 5; *Aug. 2nd*, 6; *5th*, 5; *7th*, 10; *8th*, 7; *9th*, 4; *17th*, 2; *19th*, 1. All heads failed completely as shown in fig. 1 of Plate III. There were no partially formed seeds.

Plant *R 12-11-49, no. 79*. A plant completely self-incompatible. All the pollinations here recorded were made by hand. Plant bloomed from June 21 until Aug. 13. Dates and number of heads selfed are as follows:—*June 21st*, 2; *24th*, 1; *26th*, 5; *28th*, 8; *July 2nd*, 10; *5th*, 5; *10th*, 6; *21st*, 5; *23rd*, 4; *26th*, 3; *Aug. 4th*, 8; *6th*, 3; *8th*, 1; *9th*, 1; *12th*, 1.

It is quite probable that a larger number of pollinations during the early portion of the period of bloom, or an extension of tests over a longer portion of the time of blooming, would show that plants judged as self-incompatible were somewhat feebly self-compatible. The tests made show that plants may be highly self-fertile, partially self-fertile, or completely self-sterile from the very first date of bloom. If end-season self-fertility does develop my results are in error in that plants classed as self-incompatible may later have become somewhat self-compatible. Apparently in chicory the entire range of variability in the self-fertility of individuals is seen during the first few days of the period of bloom.

II. *Evidence that self-incompatibility may lead to certain cases of embryo abortion.*

For plants of chicory that are fully self-incompatible only mere rudiments of achenes develop and the entire head which is selfed becomes shrivelled as is shown in 1, 2, and 3 of Plate III. The numbers 4, 5, and 6 of the same plate show the well filled heads containing seeds with embryos from flower heads of the same plant cross-pollinated on the same day that the heads of figs. 1 and 2 were selfed. The conditions here shown are typical of self-incompatibility, and are proof that (1) the sex-organs are potent and capable of functioning in certain relations and that (2) the plant is able to nourish embryos when there is compatible fertilization. It seems clear that embryo abortion of any sort, and especially that involving a condition of vegetative vigour, is not operating in such extreme cases of incompatibility.

For the feebly self-compatible plants, however, there is usually a rather graded series of more or less developed but empty achenes as is shown in 7 and 8 of Plate III. At *a* of no. 7, is a group of 14 mere rudiments of achenes, at *b* are 5 achenes of good size but entirely empty, and at *c* is one good achene with an embryo; these are all from a single head of a plant judged as feebly self-compatible. No. 8 shows a graded series of 16 empty achenes and four good seeds, two of which were crushed to determine if embryos were present.

No. 9 shows 11 good seed, 2 empty achenes which appear externally to be good seed, and 7 that are obviously shrivelled and empty.

Thus in the partially self-fertile plants there are usually present in those heads which have good seed some achenes which are considerably developed but which contain no embryos at maturity. Possibly the development of a few seeds has an influence on the phenospermic (using term of Goodspeed, 1915) development of certain others of the head

108 *Self-Incompatibility in Hermaphrodite Plants*

which were not fertilized. It may be, however, that in some of these phenospermic achenes embryo abortion occurs as an expression of incompatibility after fertilization. This appears also to be the case for feebly self-fertile plants of *Eschscholtzia californica* as noted above. The ability of such plants to produce abundant seed to compatible cross-pollinations at the same time that phenospermic seeds are produced among heads selfed indicates that any embryo abortion which may occur is associated with incompatibilities rather than with inability of parent to nourish young embryos. It is to be recognized however that in chicory the development of the head as a whole may in large measure depend on the number of ovules fertilized properly, and that the development of the individual seeds in a head may thus be influenced by local relations. If such is the case, the effects would be most liable to appear in plants partially or feebly self-compatible.

Considerable cytological investigation has been directed to the study of these conditions in chicory, but at the moment of writing the observations are not conclusive as to the extent to which true embryo abortion may occur. The results, however, indicate that in plants fully self-incompatible there is complete failure in fertilization due to the poor growth of pollen tubes. In plants feebly self-fertile there appear to be various stages at which the processes of fertilization may fail.

III. *The heredity of self-compatibility in self-fertilized lines of descent in the variety red-leaved Treviso of Cichorium Intybus.*

The immediate parents of this generation were highly self-fertile plants which had descended from three generations of ancestry known to have been self-sterile at least during the early part of their period of bloom. The race had been kept in culture by rather close inbreeding. This generation therefore afforded material for the study of the heredity of self-fertility after it has arisen sporadically in an inbred variety.

The number of plants grown in the I_1 , and also the I_2 , was so large that it was impossible to test all of these by controlled hand pollination (described 1916, p. 362) as was done in previous studies with this species. The plants were tested by autonomous self-pollination. Flower heads were allowed to open and close under a bag. Such heads were appropriately tagged and allowed to continue development in the open. As a rule flower heads of chicory are open but a few hours during the forenoon; by midday they are closed never to open again, and the next day the corollas and styles fall. The entire afternoon can be devoted to

shifting bags, and tagging heads. Bags need to remain enclosing a group of branches for only 24 hours. By beginning on the first or second day of bloom and shifting a bag about three times, about 30 flower heads per plant can readily be selfed during the first ten days of bloom.

The I_1 generation.

Tests for self-compatibility were made for 351 plants of the I_1 generation which were grown from the self-fertilized seed of 3 self-compatible plants. The presentation of data for all these plants would involve rather extended tables, hence complete data will here be given only for certain self-compatible plants which are fully representative of the results obtained, and illustrate the range in the percentages of self-compatibility. In the following tables dates of pollination and number of seed per head are given. The period of bloom is given for the I_2 , but for the I_1 the last dates of blooming were not recorded. The percentage fertility for all plants of the I_1 and I_2 is based on the proportion of flowers (estimated at 20 per head) which produced seeds, and the percentages for the few self-fertile plants obtained previously are here also thus determined. A general summary showing distribution according to the degrees of self-fertility is given in Table IV (p. 115).

Series R 12-11- . The immediate parent of this series was one of evidently high self-fertility (1917, Table I). It was, however, the only one of 18 sister plants which was found to be self-compatible. Of this series 35 plants were self-incompatible and 26 were self-compatible. Data for 8 of the latter are given in Table I (p. 110).

Series R 10-8- . The 177 plants of this series were grown from the selfed seed of a plant (*R 10, no. 8*) whose self-compatibility is here estimated at 55%. This plant was the only one of 10 sisters to set seed to selfing. Of the series, 128 plants were completely self-incompatible and 49 were self-compatible. The fertilities ranged to 42%.

Series R 9-34- . The immediate parent (*R 9, no. 34*) was one of 25 sister plants three of which were self-compatible. Controlled self-pollinations were made on 113 of the progeny: 81 were self-sterile and 32 were self-fertile with fertilities ranging to 43%. In this series there were also 40 plants which were highly *impotent*, a condition discussed later.

The I_2 generation.

The number of plants tested for self-compatibility in this generation was 471. The number of flower heads tagged for autonomous selfing was 14,390; the average per plant was 30 flower heads or about 200

110 *Self-Incompatibility in Hermaphrodite Plants*

TABLE I.

Typical records for plants of three series of the I₁ generation from self-compatible parentage.

Pedigree	Date of first bloom	Dates of self-pollination.	Number of seed per head	Fertility (per cent.)
<i>R 12-11-</i>				
No. 59	July 5	July 5th, 17, 18; 7th, 11, 15, 18; 9th, 3, 7, 12, 13; 10th, 5, 6, 10, 16, 19, 20; 25th, 3, 11, 14		·60
„ 51	June 29	June 30th, 9, 16, 22; July 5th, 15; 6th, 5, 12, 14; 7th, 7, 15, 18; 10th, 2, 5, 6, 7, 12, 13, 16		·56
„ 16	July 12	July 13th, 0, 3; 15th, 0, 2, 11; 16th, 6, 7, 12, 12, 13, 13, 20		·37
„ 49	July 5	July 6th, 11; 7th, 2, 9, 9, 9, 10, 13, 14; 9th, 6, 9, 10, 10, 12; 18th, 0, 2, 2, 3, 8, 9, 9, 11; 20th, 1, 5; 24th, 2, 3, 4, 8; 25th, 2, 4, 6, 7, 8, 9, 13		·35
„ 30	July 12	July 13th, 0, 0, 1, 8; 14th, 0, 0, 2, 9; 16th, 3, 7, 9, 11, 12		·24
„ 63	July 21	July 23rd, 0, 0, 0, 0, 0, 0, 1, 5, 9, 11, 12; 25th, 0, 9; 27th, 0, 0, 2; 30th, 0, 0, 0, 0, 0; 31st, 0, 0, 0, 0, 0		·10
„ 18	July 10	July 12th, 0, 0, 0; 13th, 0, 0, 0, 0, 2, 2; 14th, 0, 0, 0, 0, 3; Aug. 6th, 0, 0, 0, 0; 8th, 0, 0, 0		·02
„ 25	July 20	July 23rd, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 3; 25th, 0, 0, 0, 0, 0; 27th, 0, 0, 0, 0, 0, 0, 0, 0, 1		·01
18 other plants self-fertile 35 plants self-sterile Total flower-heads self-pollinated, 973				
<i>R 10-8-</i>				
No. 63	Aug. 4	Aug. 6th, 0, 2, 3, 3, 5, 7, 8, 9, 10, 13, 15, 16, 17, 17; 8th, 0, 3, 7, 16		·42
„ 140	July 27	July 30th, 0, 0, 0, 0, 0, 3, 7, 7, 7, 7, 8, 9, 12; Aug. 9th, 0, 0, 0, 3, 4, 5, 7, 8, 18; 10th, 0, 3, 3, 8, 13, 15, 16		·30
„ 37	July 29	Aug. 1st, 0, 0, 1, 1, 1, 1, 1, 4, 8; 3rd, 0, 12, 12, 13, 15; 6th, 0, 1, 3, 4, 9, 9, 14, 17		·29
„ 173	July 23	July 25th, 0, 0, 0, 0, 4, 10; 27th, 0, 0, 0, 0, 0; Aug. 8th, 0, 0, 0, 1, 4, 6, 7, 10; 9th, 0, 0, 0, 1, 2, 4, 5, 6, 7, 8, 11		·14
„ 20	July 21	July 23rd, 0, 0, 0, 0, 0; 27th, 0, 0, 0, 0, 0, 0, 3, 5, 6; 30th, 0, 0, 0, 0, 1		·03
44 other plants self-fertile 128 plants self-sterile Total flower heads, 1424				
<i>R 9-34-</i>				
No. 46	July 23	July 25th, 6, 10, 10, 15, 15; 30th, 0, 0, 1, 6, 6, 6, 6; Aug. 9th, 0, 0, 2, 4, 6, 6, 7, 9, 13; 13th, 3, 3, 7, 8, 8, 8, 9, 12, 12, 12, 14, 18; 15th, 1, 1, 4, 5, 9, 9, 11, 12, 13, 14; 17th, 0, 3, 4, 7, 8, 9, 10, 10, 10, 11, 12, 12		·38
„ 22	July 27	July 30th, 5, 9, 9, 10, 12, 14; Aug. 1st, 0, 0, 0, 0, 2, 5; 11th, 0, 0, 0, 0, 2, 2, 6, 6; 6th, 0, 0, 0, 0, 12; 13th, 0, 0, 0, 1, 2, 4, 5, 6, 6, 9, 11, 12, 13, 15, 16; 15th, 0, 0, 1, 9, 10; 10th, 0, 0, 3, 3, 5, 6, 8, 9, 9, 11		·23
„ 88	July 21	July 23rd, 0, 0, 0, 0, 0, 2, 3, 8, 8; 25th, 0, 0, 0, 2, 3, 3, 4, 7, 8; 27th, 0, 0, 0, 0, 0, 1, 1, 1, 2, 2, 6		·10
„ 73	Aug. 19	Aug. 20th, 0, 0, 0, 0, 4, 4; 22nd, 0, 0, 0, 2, 3, 5; 24th, 0, 0, 0, 0, 0, 0, 0, 2; 27th, 0, 0, 0, 0, 0, 5, 8		·05
„ 91	Aug. 13	Aug. 14th, 0, 0, 0; 15th, 0, 0, 0, 0, 2, 4; 17th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1		·01
27 other plants self-fertile 81 plants self-sterile 40 plants impotent Total flower heads, 2643				

individual flowers. This generation descended from 6 of the I_1 generation and these in turn from 2 plants of the 1916 crop. The complete pedigree is given in Table IV and the records for typical plants of the two families of the I_2 generation are given in Tables II and III (pp. 112—113).

The family *R 10-8-*

Series R 10-8-173- . The parent of this series (see Table I) was judged as 14 % self-compatible. Of the 84 seed planted, 66 germinated and 55 plants were grown to maturity and tested. Thirty-six plants were self-incompatible and 19 were self-compatible, a ratio of 2 to 1. The highest percentage of self-compatibility was 47 %.

Series R 10-8-37- . The immediate parent was judged as 29 % self-compatible. Of its seed, 126 were planted; 86 seed germinated and 56 plants were grown to maturity and tested. Thirty-nine plants were self-compatible and 17 were self-incompatible, a ratio the inverse of that in the series above. The percentages of self-fertility for 4 plants were above 50 and there were 11 plants with a percentage higher than 25.

Series R 10-8-140- . The self-compatibility of the parent was judged to be 30 %. Mice destroyed many seed after planting but 49 plants were grown and tested. Of these 32 were self-compatible.

The family *R 12-11-*

Three series were grown in the I_2 of this family. The immediate parents were judged as 60, 37, and 35 % self-compatible from data presented in Table I.

Series R 12-11-59- . Of 175 seed planted, 155 germinated, of which 136 plants were grown and tested. Forty-two were self-incompatible and 94 were self-compatible. Three plants evidently feebly self-compatible were prematurely killed by pine mice; the individual fertilities of the others ranged to 59 % with distribution as shown in Table IV. Complete data for 3 plants of this series have already been given and discussed on page 106.

Series R 12-11-49- . The immediate parent was judged as 35 % self-fertile. Of the 210 seed planted, 136 germinated, and 131 plants were grown to maturity. Tests for self-fertility were made for 115 plants; 77 were self-incompatible and 38 were self-compatible to some degree. There was only one plant with a self-fertility above 25 %.

Table III, in which the results for three of the self-fertile plants of this series are given, includes also certain special data. That for *No. 12*

112 *Self-Incompatibility in Hermaphrodite Plants*

TABLE II.

Typical records for plants of the family R 10-8- in the I₂ generation of self-compatible parentage.

Pedigree	Period of bloom	Dates of pollination.	Number of seed per head	Fertility (per cent.)
<i>R 10-8-173-</i>				
No. 26	July 23—Oct. 15	July 25th, 0, 0, 0, 5, 12, 13, 14, 15, 18; 29th, 0, 0, 6, 14, 15, 15, 15, 16, 17, 17; Aug. 1st, 5, 5, 5, 6, 9, 13		·47
„ 30	July 17—Oct. 12	July 18th, 0, 5; 25th, 0, 1, 2, 2, 2, 2, 3, 3, 6, 6, 8; 29th, 0, 5, 6, 6, 11, 11, 17; Aug. 1st, 0, 3, 5, 7, 9		·24
„ 35	July 22—Oct. 15	July 25th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 7, 7, 7, 9, 9, 9; 29th, 0, 0, 0, 0, 0, 0, 0, 0, 2, 4, 7, 8		·12
„ 22	July 18—Oct. 10	July 18th, 0, 0; 20th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 9; 24th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 2, 1		·02
„ 1	July 27—Nov. 1	July 29th, 0, 0, 0, 0, 0, 0; Aug. 1st, 0, 0, 0, 0, 0, 0; 3rd, 0, 0, 0, 0, 0; 6th, 0, 0, 0, 0, 0, 0; 8th, 0, 0, 0, 0, 0, 0, 0, 0		·00
14 other plants self-fertile				
36 „ „ self-sterile				
Total number flower heads selfed, 1526				
<i>R 10-8-37-</i>				
No. 85	July 24—Nov. 1	July 26th, 3, 9, 10, 14, 14, 14, 15, 15, 16, 16; 30th, 0, 3, 4, 7, 7, 8, 8, 9, 10, 10, 11, 12, 13, 16, 20		·53
„ 71	July 15—Nov. 3	July 16th, 10, 10, 13, 14, 15, 15, 15; 23rd, 1, 3, 3, 3, 4, 5, 7, 7, 7, 8, 8, 9, 10, 10, 12, 13, 13, 13, 13, 13, 14, 15, 20		·50
„ 28	July 26—Nov. 4	July 26th, 0, 1; 29th, 0, 0, 0, 2, 3, 3, 4, 6; Aug. 2nd, 0, 0, 0, 1, 1, 1, 1, 1, 2, 2, 3, 3, 4, 5, 5		·09
„ 53	July 28—Nov. 1	July 30th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1; Aug. 1st, 0, 0, 0, 0, 0, 0, 1, 1, 1, 1, 1, 2, 2		·02
„ 25	July 25—Oct. 5	July 26th, 0, 0; 29th, 0, 0, 0, 0, 0, 0, 0, 0, 0; Aug. 2nd, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0; Aug. 5th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0		·00
34 other plants self-fertile				
17 „ „ self-sterile				
Total number flower heads, 1555				
<i>R 10-8-140-</i>				
No. 19	July 27—Nov. 1	July 31st, 1, 3, 4, 4, 5, 6, 6, 7, 7, 11, 12, 15, 16, 17, 19; Aug. 3rd, 4, 9, 10, 10, 11, 13, 15, 15, 16, 16, 18		·52
„ 42	July 26—Nov. 6+	July 28th, 0, 0, 2, 7, 7, 10, 12, 15, 15; 31st, 0, 0, 0, 0, 2, 5, 7, 7, 8, 11, 11, 12, 13, 13, 13, 16		·38
„ 31	July 18—Oct. 15	July 18th, 0, 2; 24th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 1, 1, 1, 2, 2, 3, 3, 3, 4, 4, 4, 5, 5, 6, 9; 27th, 0, 0, 0, 0, 1, 3, 4; 31st, 0, 0, 2, 5, 6, 8		·10
„ 6	July 22—Nov. 6+	July 24th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 2, 2, 7; 27th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 3, 3, 3, 4, 6		·05
„ 2	July 17—Nov. 1	July 18th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0; 24th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0; 27th, 0, 0, 0, 0, 0, 0, 0; 31st, 0, 0, 0, 0, 0		·00
27 other plants self-fertile				
17 „ „ self-sterile				
Total number flower heads, 1393				

TABLE III.

*Typical records for plants of the family R-12-11- in the I₂ generation
of self-compatible parentage.*

Pedigree	Period of bloom	Dates of pollination.	Number of seed per head	Fertility (per cent.)
<i>R 12-11-59-</i>				
No. 95	July 8—Sept. 4	July 9th, 5, 12, 19, 19; 12th, 9, 10, 11, 11, 12, 14, 14, 14, 15, 15, 17, 18; 16th, 4, 6, 8, 9, 10, 11, 13; 22nd, 12, 13		59
„ 4	July 10—Sept. 10	July 12th, 8/18, 14/18, 12/20, 13/21, 14/22, 17/24, 17/25, 18/26, 19/26; 17th, 7/18, 7/18, 10/18, 1/19, 4/19, 6/20, 7/21, 11/22, 3/24, 16/26; 18th, 1/18, 1/18, 3/18, 7/18, 1/19, 3/19, 6/19, 12/19, 15/20, 11/21, 14/22, 16/24; 22nd, 9/16, 2/18, 15/18, 16/20, 16/20, 16/24		41
„ 1	July 7—Sept. 2	July 8th, 0, 6/18, 4/19; 12th, 0, 0, 0, 0, 2/16, 8/16, 2/17, 6/17, 9/17, 2/18, 9/18, 11/21; 16th, 0, 0, 0, 3/16, 5/17, 5/18, 4/19, 5/19, 9/21; 22nd, 0, 3/17, 12/17, 6/18, 13/19, 11/20; 25th, 0, 0, 5/15, 12/16, 13/17, 5/18, 6/18, 7/18, 2/20		23
91 other plants self-fertile 42 plants self-sterile Total number flower heads selfed, 4034				
<i>R 12-11-49-</i>				
No. 12	July 10—Aug. 29	July 11th, 7/17, 13/18, 15/19, 6/20; 16th, 7/16, 10/16, 11/16, 1/17, 6/17, 9/17, 12/17, 13/17, 14/17, 5/18, 8/18, 8/18, 10/18, 10/18, 12/18, 13/18, 13/18, 9/19, 13/19, 2/20, 9/20, 10/22		49
„ 80	July 6—Sept. 12	July 8th, 0, 0, 0, 0; 11th, 0, 0, 0, 2, 5, 6, 9, 9, 10; 16th, 0, 0, 3, 4, 5, 5, 5, 6, 6, 6, 7, 8, 10, 10, 10, 12		23
„ 33	July 16—Sept. 11	July 16th, 0, 0, 0, 0, 8; 18th (hand), 0, 0, 0, 0, 0, 1, 4, 10; 20th (hand), 0, 0, 1, 3, 3, 5, 8; 23rd, 0, 0, 0, 0, 0, 0, 0, 0, 3, 3, 7, 8, 8, 10		10
„ 50	July 8—Sept. 8	July 8th, 0, 0; 10th, 0, 0, 0, 0, 0, 0, 0, 3, 4, 10; 15th, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0; 23rd, 0, 0, 0, 0, 0, 4; (23rd, cross-pollinated, 10/16, 12/16, 13/17, 12/18, 14/18, 14/18, 18/18, 15/19, 16/19, 16/19); 26th, 0, 0, 0, 5, 10; 31st, 0, 0, 0, 0, 0		04
34 other plants self-fertile 77 plants self-sterile Total flower heads selfed, 4106				
<i>R 12-11-16-</i>				
No. 37	July 13—Sept. 12	July 15th, 10/18, 13/18, 11/19, 15/22, 18/23, 14/24, 15/25; 16th, 13/17, 13/18, 5/19, 7/20, 5/21, 17/22, 18/22, 10/23, 15/23, 18/23, 20/23, 20/23, 15/24, 23/24, 19/25, 24/25; 18th (hand), 12/19, 14/19, 17/19, 7/21, 16/21, 20/21, 15/22, 19/24, 17/25, 20/25; 24th, 13/18, 14/18, 12/19, 17/20, 16/21, 14/22, 19/23, 15/24		75
„ 11	July 8—Sept. 4	July 10th, 9, 11, 12, 12, 14; 15th, 4, 4, 5, 6, 7, 7, 7, 9, 11, 12, 12, 13, 14, 14, 14, 15, 18, 20; 19th, 6, 8, 9, 12, 12, 15, 16; 24th, 8, 11, 12, 12, 12, 14, 15		56
„ 58	July 8—Sept. 10	July 10th, 2, 2, 10, 13, 15; 15th, 1, 1, 3, 3, 3, 3, 4, 5, 5, 6, 8, 10, 11, 13, 13, 14, 15; 19th, 1, 2, 4, 6, 8		33
„ 60	July 5—Sept. 8	July 8th, 0, 0, 0, 0, 0, 5, 6, 6, 7, 9; 10th, 0, 0, 0, 0, 1, 3, 3, 5, 5, 8; 15th, 0, 0, 0, 0, 0, 0, 2, 3, 4		11
40 other plants self-fertile 16 plants self-sterile Total flower heads selfed, 1776				

114 *Self-Incompatibility in Hermaphrodite Plants*

gives the number of flowers per head expressed as a denominator and the number of seed obtained as the numerator of a fraction. (Such data show that the variations in number of seed per head are quite independent of the number of flowers per head.) Data for *No. 33* give comparative results of hand and autonomous self-pollination, which are typical of the evidence that careful self-pollination by hand does not increase self-fertility over autonomous selfing.

The data for *No. 50* include for comparison the results of the controlled cross-pollinations of 10 heads, and are quite typical of the abundant evidence obtained which shows that feebly self-compatible or strongly self-incompatible plants are highly productive of seed to compatible crosses made on the same dates of bloom.

Series R 12-11-16- . All of the 103 seed obtained from the self-pollinations of the parent were planted. Sixty-nine germinated, but possibly as many as 20 embryos had been injured at the time seed was examined to determine if embryos were present. Of the 60 plants tested for self-fertility, 16 were self-incompatible and 44 were self-compatible.

The data given for *No. 37* in Table III include results of self-pollinations by hand for 10 heads, and for all heads the numbers of flowers and seeds are expressed in the form of a fraction.

General summary. Table IV presents a grand summary of the various crops of the variety red-leaved Treviso with the pedigree indicated and the distribution of the individual fertilities given. As there shown the variety was kept in culture for the first three years (1914, 1915, 1916) by crossing self-incompatible plants. The first plant found to be self-fertile was one of the 1915 crop but it was very feebly self-fertile and the two offspring grown from its seed were self-incompatible. Among the 1916 crop grown from self-incompatible parentage, there were 11 plants which were self-fertile to some degree and of these 4 were highly self-fertile.

The subsequent generations descended from self-fertile plants, and the study of fertilities in them constitutes a test for the heredity of self-compatibility that arose sporadically after three generations of self-incompatible parentage. Three lines were grown in the I_1 , and two families were continued into the I_2 .

The proportion of plants that were self-fertile was decidedly increased in the I_1 and was still larger in the I_2 . In both families of the I_2 there was also an extension in the range of self-fertilities.

The two families grown into the I_2 exhibit some differences in self-

TABLE IV. Records of self-compatibility for families, lines of descent and series of the variety red-leaved Treviso.

Record of Offspring

Record of Ancestry			Percentage self-fertility of		No. Plants		Distribution. Percentage fertility																
Parentage			P_1	P_2	Total	Self-sterile	Self-sterile fertile	Percent-sterile															
Com. seed ...									1-5	-10	-15	-20	-25	-30	-35	-40	-45	-50	-55	-60	-65	-70	-75
1913	Com. seed	...	—	—	10	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1914	(19 × 27) of 1913	...	—	—	11	11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1915	Ser. 1. (1 × 2) of 1914	...	—	—	10	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"	4. (8 × 6)	"	—	—	8	7	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"	5. (8 × 2)	"	—	—	20	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1916	6. Ser. 4, no. 21	...	—	—	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"	7. Ser. 1 8 × 5	5	—	—	10	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"	8. " 4 1 × 5	8	—	—	19	18	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
"	9. " 5 1 × 5	6	—	—	25	22	3	—	?	—	—	—	—	—	—	—	1	—	—	1	—	—	—
"	10. " 4 12 × 5	9	—	—	10	9	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
"	11. " 5 1 × 1	8	—	—	19	14	5	—	4	1	—	—	—	—	—	—	—	—	—	—	—	—	—
"	12. " 5 9 × 4	18	—	—	18	17	1	—	—	—	—	—	—	—	—	—	—	—	—	—	?	—	—
1917	I_1 , Ser. 9-34	...	56	—	113*	81	32	28	12	5	5	4	2	1	1	2	—	—	—	—	—	—	—
"	" 10-8-	...	55	—	177	128	49 ²	28	6	11	7	9	6	6	1	0	1	—	—	—	—	—	—
"	" 12-11-	...	70?	—	61	35	26	43	6	7	1	1	2	0	2	4	0	1	0	2	—	—	—
Summary for I_1			—	—	351	244	107	30	24	23	13	14	10	7	4	6	1	1	0	2	—	—	—
1918	I_2 , Ser. 10-8-173-	...	55	14	55	36	19	35	1	6	2	6	2	0	0	0	1	1	—	—	—	—	—
"	" 10-8-37-	...	55	29	56	17	39 ¹	70	4	9	3	8	3	1	1	2	2	1	4	—	—	—	—
"	" 10-8-140-	...	55	30	49	17	32	65	8	6	2	4	2	3	2	2	0	2	1	—	—	—	—
Summary for Fam. 10-8-			—	—	160	70	90	56	13	21	7	18	7	4	3	4	3	4	5	—	—	—	—
1918	I_2 , Ser. 12-11-59-	...	70?	60	136	42	94 ³	69	15	13	15	13	9	12	3	4	4	1	1	1	—	—	—
"	" 12-11-49-	...	70?	35	115	77	38	33	10	8	8	8	3	—	—	—	—	1	—	—	—	—	—
"	" 12-11-16-	...	70?	37	60	16	44	70	1	4	4	5	5	7	7	2	0	3	1	2	1	1	1
Summary for Fam. 12-11-			—	—	311	135	176	56	26	25	27	26	17	19	10	6	4	5	2	3	1	1	1
Summary for I_2			—	—	471	205	266	56	39	46	34	44	24	23	13	10	7	9	7	3	1	1	1

¹ Percentage not determined for one, ² for two, ³ for three self-fertile plants. * 40 other plants impotent not considered in percentages.

¹ Percentage not determined for one, ² for two, ³ for three self-fertile plants. * 40 other plants impotent not considered in percentages.

116 *Self-Incompatibility in Hermaphrodite Plants*

fertility. In the I_1 , Series 12-11 was more self-fertile both as to proportion of plants and range of self-fertilities. In the I_2 , considering each family as a whole, there was no difference in the proportion of plants, but the ranges were higher for one series of the family 12-11- .

Some irregularities are apparent in which the behaviour of offspring is not directly to be gauged by the degree of self-fertility of a parent. In the I_1 of family 10-8- one series (10-8-173) gave a ratio of 2 self-sterile to 1 self-fertile while in the other series this ratio was reversed; the grades of self-fertility for the three immediate parents were almost identical and they all had the same ancestry preceding. In the I_2 of the family 12-11-, the two series were obtained from parents of almost identical self-fertility; one of these (12-11-49) was of decidedly feeble self-fertility in comparison with the other (12-11-16).

IV. *Vegetative vigour and impotence in self-fertilized lines in the variety red-leaved Treviso of Cichorium Intybus.*

The various grades of seed development and embryo abortion in plants feebly self-compatible suggest strongly that individuals of various grades of vegetative growth may arise through various grades of compatibility in the fertilizations. The physiological basis for degeneracy in individuals or races may thus be sought, in part at least, in the comparative weak compatibility of the elements which unite in fertilization. That this is the case is also suggested by the evidence that inbreeding and continued self-fertilization is not of itself injurious.

The self-fertilized lines of descent in this variety of chicory were derived from a race that was kept in culture for three years by cross-fertilization of rather closely related plants. Undoubtedly the variety had previously been maintained largely by crossing, and it is a salad chicory that has been developed and maintained by selection for vigorous vegetative growth. Under such treatment there has been a constant selection of the offspring of highly compatible fertilizations and an elimination of weak individuals which in my culture have been given a chance for their complete development.

In the cross-fertilized stock of the first three years of culture there were occasional plants that exhibited signs of degeneracy. The leaves and branches were few and poorly developed, flower heads did not open fully, corollas were crumpled, and many anthers were dark coloured, contained few well-developed pollen grains and often did not dehisce. Such plants produced few seed to open cross-pollination. During the

first few years in my culture plants of this variety ranged from $4\frac{1}{2}$ to $6\frac{1}{2}$ feet in height and were quite uniform in general appearance. The occasional degenerate plant was always one of the smaller plants. No controlled pollinations were made for such plants and they were not included with self-incompatible plants.

It has also been observed that abnormalities may be present in the pollen giving grains of noticeably large size. Cytological preparations show that these giant pollen grains are due to the incomplete separation of the four daughter cells that result from the reduction division. Quadripartition is arrested and a spore wall is formed about all four daughter nuclei. In some cases the giant grain is decidedly lobed. Such irregularities in the development of pollen have not been observed in other stocks (wild, Barbe de Capucin, and hybrid generations) in which examination of much pollen has been made. The proportion of giant grains has not been large and they have been found in largest numbers among highly self-compatible and incompatible plants indiscriminately. For many self-incompatible plants, giant grains were absent or rare. Thus it would seem that their development has no connection with incompatibilities, but is rather associated with the conditions of duplication and cohesion which are characteristic of this variety as described elsewhere (1918b).

The three highly self-compatible plants selected as parents of the I_1 gave progenies that were decidedly different in regard to vegetative vigour and potentiality for seed production. One line of descent was decidedly degenerate, the others were highly vigorous in growth and in sex vigour.

1. *A degenerate line of descent.* One of the parent plants, *R 9 No. 34*, was $4\frac{3}{4}$ feet tall, well branched and exhibited no signs of degeneracy. Its main axis was strongly duplicated and there were lesions and considerable torsion quite as illustrated in a former paper on this type fasciation (see No. 9 of Plate XII, 1918b). Giant pollen grains were frequent, but the plant was decidedly self-compatible and set abundant seed to open pollination.

The series (*R 9-34-*) grown from its seed was noticeably low in vegetative vigour. No plant was more than $5\frac{1}{2}$ feet tall; few were more than 5 feet; numerous plants were only from 3 to $4\frac{1}{2}$ feet in height and some were but 2 feet tall. Many plants produced very few seed to open pollination and some produced no seed at all. Such impotent plants, about 40 in number, were not included in the self-incompatible plants recorded for this series.

118 *Self-Incompatibility in Hermaphrodite Plants*

For the I_2 of this line the progeny of one plant, *R 9-34 No. 46*, were grown. This plant was the most highly self-compatible, and was one of the best developed plants of its series. The flower heads opened normally and the anthers dehisced properly. Of the 240 seed sown only 160 germinated. Many seedlings were poorly developed; 32 died within four weeks after germination; 18 others died before the crop was planted in the field.

Of the 111 plants grown in the field only three were over $4\frac{1}{2}$ feet tall; 60 were from 8 inches to 3 feet in height when fully mature. Flower heads developed and opened poorly on nearly all plants.

At least 50 set no seed at all, and only 6 plants produced seed in considerable numbers. Giant pollen grains were found for 13 out of 30 plants whose pollen was examined. All plants were fasciated in some degree, and in the majority the duplication was strong with much torsion, but lesions were not frequent.

The series of the I_2 was characterized by extreme degeneracy in vegetative growth and sexual potentiality. This condition was more marked in the I_2 than in the I_1 , both as to the degree to which individual plants were degenerate and as to the proportion of such plants.

2. *Families of marked vegetative vigour.* No case of degeneracy either in vegetative growth or in potency for seed production developed in the other two lines of descent of the I_1 . The parents were well developed plants having slight or medium degrees of fasciation. One parent *R 10 No. 8*, shown in plate of a former paper (Stout 1917, Fig. 1), was one of the tallest and most vigorous plants grown to that date. The other parent (*R 12 No. 11*) was a smaller plant and bloomed for a shorter period.

All plants of both series were highly productive of seed to open pollination. Some irregular or giant pollen grains were found, but these were present for self-compatible and self-incompatible plants without discrimination. The series *10-8-* was taller in growth, and began bloom later but bloomed for a longer period than did series *12-11-*. Each series was rather uniform in itself and the differences between them were identical with those exhibited by their respective parents.

For the I_2 generation three series in each family were grown. In all, there were 471 plants whose self-compatibility was determined and 25 others whose fertility was not studied. Every plant produced flowers which appeared to be fully normal, all were highly productive of seed to open pollination, and all were of vigorous growth.

The differences in vegetative growth of these two families seen in

the I_1 were continued into the I_2 . Series 10-8-37 and 10-8-140 especially were of vigorous growth. Fifty of the 56 plants of series 10-8-37 were 6 or more feet tall and a few were 7 feet tall. The majority of the plants of 10-8-140 were from $5\frac{1}{2}$ to $6\frac{1}{4}$ feet tall. (See Plate IV at right with field label 26.) In these series were the largest, tallest and most vigorous plants that have thus far been grown in my cultures. The plants of series 10-8-173 were of somewhat smaller stature.

The three series of the I_2 of the family 12-11- were remarkably uniform in habit of growth as is well shown in Plate IV. The height scarcely varied more than 6 inches. Some plants in each series began blooming in June and were about 10-15 days earlier in blooming than plants of the family 10-8- .

Line breeding with self-fertilization for two generations has thus led to the isolation of families or lines differing in general vegetative vigour.

One line continued to the I_2 exhibited a decided degeneracy which became more marked in the I_2 than in the I_1 . There may be some question as to whether this was due to some heritable factor of constitutional organization such for example as extreme conditions of fasciation, or whether a relation in sexual reproduction is operating as an immediate cause, as it appears to be in many degenerate hybrids and in the quite similar degenerative offspring of certain illegitimate and weakly compatible matings reported in *Lythrum Salicaria* (Darwin 1869, 1877).

Two main lines maintained in both the I_1 and the I_2 a high degree of vegetative vigour and potential sex vigour, and one of these has seemed to gain in vegetative vigour over that of the parent stock. The uniformity of these differences here suggests that constitutional and heritable "factors" for size are present, and are not perceptibly influenced by such variations in self-compatibility as may have occurred in the rather highly self-compatible parents.

The readiness with which self-fertilized lines from parentage of high self-compatibility maintain a high degree of vegetative vigour is convincing evidence that self-fertilization is of itself not directly injurious and productive of degeneracy.

DISCUSSION AND CONCLUSION.

It seems clear that both self-fertility and cross-fertility within a species are original and primitive conditions as compared with self-incompatibility and cross-incompatibility. Further it seems clear that self-fertility is more primitive than cross-fertility just as hermaphroditism is obviously the more primitive condition out of which dioecism has developed. In hermaphrodites incompatibilities have arisen in species, and evidently are arising at the present time, through fluctuating variation in the physiological differentiation of the sex organs. These phenomena run parallel to the anatomical variations leading to intersexualism and dioecism.

The evidence supporting this general conclusion from my own studies and from the facts revealed by other recent studies as well may here be summarized under the following heads:

1. The indisputable evidence that compatibility and incompatibility in many species are highly variable both in expression and in heredity.

2. The evidence is conclusive that self-incompatibility is not always, if ever, induced by self-fertilization and inbreeding.

3. Variations, now recognized as phenomena of intersexualism, in morphological sex differentiation in species which are prevailingly hermaphrodite or dioecious, are quite analogous to variations in physiological differentiation.

4. The obvious conclusion is that sex differentiation and determination, and hence compatibility and incompatibility in hermaphrodites, are fundamentally of ontogenetic and biogenetic development.

1. The evidence is conclusive that in the various so-called self-incompatible homomorphic species there are individuals that are to some extent self-compatible, and that there is among these wide variability as to the number of sex organs that will function together. This is true at least of nearly all species whose self-fertility has been studied. The same general conditions are found in cases of cross-incompatibility within such species. Even reciprocals between pairs of plants may give opposite results.

As a rule feeble or partial compatibility manifests itself quite indiscriminately throughout the entire period of bloom. Marked cases of a periodic change in compatibility do occur such as end-season self-fertility (in *Nicotiana Forgetiana* Hyb. Hort. and in *Lythrum*

Salicaria) and mid-season self-fertility (in *Brassica pekinensis*) but from the evidence at hand these seem to be characteristic of certain individuals rather than a condition regularly present in the species. Such partial variations may be regarded as reversion to the more primitive condition. They exist along with other wide variations in the degree to which self-compatibility operates.

Not only is there variation in the number of sex organs that function together, but there appears to be considerable variation as to the stage to which processes of fertilization proceed, and also even in the vigour of the seed from fertilizations that are successful. Observations by many investigators confirm the fact that in some cases the pollen tubes make such feeble growth that fusion of gametes is not possible (see especially the recent account by East and Park, 1918). In the case of feebly self-fertile plants (chicory, California poppy, and perhaps the apple, show this very well) many poorly developed seeds stand as intermediates between the few good seeds and the mere rudiments, and suggest that some embryo abortion occurring in plants showing feeble self-compatibility may be due to certain grades of incompatibility.

As reported above in chicory, occasional plants and certain lines appear which exhibit decided degeneracy. One such family of chicory has been studied in detail. It showed grades of vegetative degeneracy, viability of seeds containing embryos was low, many plants were weak, small and short lived and many of those that lived were entirely impotent in respect to the development of stamens and pistils. Such conditions certainly suggest that the poor development of offspring may in such cases be an expression of compatibilities between the sex elements and may thus closely parallel the conditions of poor vegetative and sex vigour observed in certain, though of course not all, hybrids. The condition in this one family of chicory is quite like that reported by Darwin (1869, 1877) for the offspring of illegitimate crosses in the trimorphic species *Lythrum Salicaria*. Still it is to be recognized that we have no proof that degenerate plants or strains are more frequent in species which show self-incompatibility than in those that do not.

As reported above the heredity of self-compatibility and self-incompatibility has been specially studied in an inbred variety of chicory, and this problem had previously been studied (1918a) in the progeny of inter-varietal crosses. The sporadically occurring self-compatible plants were made the beginning of selfed lines of descent, which in certain lines have been continued for three generations. The results obtained during the seven years, during which the self-fertility of over 2,000 plants

122 *Self-Incompatibility in Hermaphrodite Plants*

was determined by controlled self-pollinations involving over 30,000 flower heads and about 600,000 individual flowers, show conclusively that self-compatible plants occur sporadically among the progeny of self-incompatible plants, and that self-incompatible plants continue to appear among the offspring of self-compatible plants. Either self-compatibility or self-incompatibility can arise from the other in a line of inbred or selfed progeny; in this sense the two conditions are reversible. Since this is the case, dominance or recessiveness of these characters cannot be adequately determined.

None the less there is a tendency to heritability in these characters. Self-fertile plants appear to constitute from 0 to 10 % of the progeny of self-sterile parents. The proportion of such plants immediately increases in the I_1 ; in this generation of the red-leaved Treviso numbering 351 plants (Table IV), 30 % of the plants were self-fertile to some degree. In other families the percentage was higher (Table VIII, 1918). The offspring of self-fertile plants are more likely to be self-fertile by a proportion of about 5 to 1. This proportion can be changed only slightly by the selection of parents of different degrees of self-fertility.

Certain lines and families appear to maintain somewhat different grades of self-fertility. This is most marked in respect to the range of individual self-fertility. All families agree in general behaviour as to regression, but some are more highly self-fertile than others. The character of self-compatibility is one of incomplete heredity; a self-fertilized line of descent does not breed true; in pedigreed lines of descent the characters self-fertility and self-sterility are reversible.

From the standpoint of a factorial description of the results obtained in chicory, several points are of significance. The spontaneous occurrence of self-compatible plants after several generations of self-sterile parentage suggests the phenomenon of mutation referable to single factors or to the recombination of modifying multiples. But submitted to the test of self-breeding the character of self-fertility does not breed true, hence any particular factors or combinations that may be assumed are not stable. The frequency distribution for fertilities suggests variation that is often interpreted in terms of multiple factors of quantitative and modifying values, and this is also suggested by the evidence that certain families exhibit somewhat different degrees of self-compatibility. Yet in all families there is marked regression to self-sterility. Perhaps the most significant fact from the standpoint of hereditary analysis is that the proportion of self-fertile to self-sterile plants in the progenies of self-fertile plants seems to fluctuate about a 1 : 1 ratio. This

simulates a condition of so-called enforced hybridity or heterozygosity involving selective elimination of the homozygotes. In making such a ratio, however, many grades of self-fertility are classed together. The experimental evidence that such ever-sporting races of inbred lines of descent are really hybrids is often obtained by the very questionable method of crossing with some other race. Continued variability and reversibility of characters in inbred lines are best interpreted as marked deviations in quantitative values or potencies of the ultimate units which Mendelian analysis and description may give. In fact such variations are very generally recognized by students of heredity.

2. Incompatibilities do not arise in species as a condition induced by self-fertilization and inbreeding.

The question as to the cause of variability in the compatibilities in hermaphrodites, and of the origin of sexual incompatibilities and their significance in evolution, involves in some measure at least the more immediate question of their relation to inbreeding and cross-breeding, and of the relative fertility of hermaphrodites of self-bred and cross-bred parentage.

It seems necessary to reiterate that Darwin was consistent in his repeated interpretations that what he called self-sterility (the type due to physiological incompatibility) is an incidental and sporadic condition arising from the influence of environment on the constitution of the sex elements. He specifically rejected (1876, p. 345) the view that such a condition arises through physiological results of inbreeding, or that it involves a fundamental necessity for cross-fertilization. He did not consider that it is a condition acquired for the special advantage of preventing self-fertilization.

Darwin held that the physiological conditions operating in the self-sterile plant involve a lack of differentiation; the sex organs were considered to be too much alike in constitution. Most writers have sought to explain self-incompatibility on this basis; either on the basis of similarity of cytoplasmic constitution (Morgan, 1904, 1910), or of hereditary units of germ plasm either of direct influence (Correns, 1912), or of indirect influence (East, 1915), or of hereditary value in transmission but cytoplasmic in the immediate relations of fertilization (East and Park, 1918). East and Park have expressed the view that cross-incompatibilities at least are decidedly increased by inbreeding.

In considering the fertility of any stock one readily recognizes with Darwin (1876, p. 312) that there are involved (1) the production of perfectly formed sex organs and (2) the relative functioning of the organs

124 *Self-Incompatibility in Hermaphrodite Plants*

formed. The latter is especially involved in what Darwin calls "relative fertility," the former is especially represented in "innate fertility." An extreme type of loss of relative fertility is seen in self- and cross-incompatibility of plants of such species as *Eschscholtzia californica*, *Nicotiana Forgetiana* and *Cichorium Intybus*.

In regard to the fertility of cross-bred stock we now have the statement of East and Park (1918, p. 527) that "neither Shull nor East has maintained that crossing increases fertility. The number of flowers and fruits is often increased but no data have appeared which indicate a decreased percentage of non-functional gametes." East, who has had wide experience in studies of inbred and cross-bred stock, thus considers that increased seed production observed in certain cross-bred progeny is due solely to an increase in the number of sex organs. If this be true it appears that heterosis, at least in species in which no compatibilities are already in evidence, does not influence compatibilities of sex organs and does not raise relative fertility, a conclusion fully reached by the more recent report by Jones (1918).

One may well hesitate therefore before assuming that any observed case of decreased productivity in selfed stock involves an actual loss in relative fertility. Such cases may involve solely the number of sex organs produced and the perfection of their development, as appears to be the condition in certain inbred strains of corn.

At the present time there is no direct or even conclusive indirect evidence that physiological incompatibility with selective fertilization (at least selective for definite hereditary units) and variations in relative fertilities arise in a species as a direct result of inbreeding and repeated self-fertilization. There is therefore some question regarding the validity of the old doctrine that there is a tendency inherent in hermaphrodites for the sex organs to be non-functional together simply because they are produced by a single individual and that foreign pollen is naturally prepotent. The accumulation of evidence that inbreeding is not necessarily injurious has led to the view that decreased vegetative and reproductive vigour in inbred stock is due to an inherently weak constitution existing before inbreeding was begun (see especially Jones, 1918). This of course still leaves the question open as to the sources and causes of the cases of weak constitution which do appear in all sorts of species.

A question of special consideration is whether inbreeding and self-fertilization in species (homomorphic) in which incompatibilities are already in evidence lead to an increase of incompatibilities. According

to a conception of multiple factors directly concerned with the transmission and expression of incompatibilities this must necessarily be a result in inbreeding or line-breeding. East and Park (1918) assume that this is true for cross-incompatibility. The evidence from chicory shows conclusively that repeated self-fertilization in line-breeding does not lead to an increase of self-incompatibility. The average self-fertility of a race may be maintained very uniformly under repeated self-fertilization. No tests for cross-incompatibility were made in those families of chicory for which new data are reported above. It may be stated, however, that all the self-incompatible plants produced an abundance of seed to open cross-pollination which could only have been between sister or closely related plants of the variety red-leaved Treviso.

Thus far the studies of self- and cross-incompatibilities have been in species in which the incompatibilities were already present as a character variable in constitutional or genetical value. No one has observed the origin of such a condition in a species. No one has produced such a condition experimentally. Numerous excellent studies have been made (see especially Kraus and Kraybill, 1918) of the influence of various conditions of nutrition on vegetative and reproductive vigour. Plants of highly self-fertile species have been rendered sterile and fruitless but in such cases the plant was fully sterile. It not only failed to set fruit to self-pollination but to all cross-pollination as well. The sterility was not relative, it was indiscriminate and absolute.

The evidence therefore that conditions of incompatibility are not directly induced by repeated self-fertilization, and are not to be ascribed to the condition of hermaphroditism as such, is further proof that variation is operating in the physiological sex differentiation of sex organs.

3. Variations in morphological sex differentiation, especially recognized as phenomena of intersexualism, occur frequently in species prevailingly either hermaphrodite or dioecious, and are quite analogous to those variations in physiological differentiation revealed by incompatibilities.

Concerning the relation between seed-sterility from incompatibility and sterility from various types of impotence there is much need of further information. In general the two classes are distinct. Incompatibility operates between sex organs either of the same hermaphrodite or of different individuals which are highly functional in certain relations. It is characteristic of self-incompatible and cross-incompatible plants that the respective sex organs may be fully developed and potent.

Many cases of pollen and embryo sac development are associated

126 *Self-Incompatibility in Hermaphrodite Plants*

with hybridity. It is well known that hybrids exhibit all grades of vegetative vigour and sexual potency. Here the wide variations in either vegetative or sexual development, or in both, indicate that certain incompatible combinations of protoplasmic elements fail to give harmonious development of the zygote. In a few cases and especially where a character is vitally concerned with nutrition (as for example the non-chlorophyll condition) there is evidently a selective death of zygotes homozygous for this character (see Belling, 1918) but as a rule degeneration and impotence in hybrids seem to result from degrees of dissimilarity in the relative constitutional organization and development inherent in the respective parents.

It is to be recognized that various grades of impotence may develop in a good species through such variations in morphological sex differentiation as are described by the term intersexualism. Intersexualism differs from impotence in hybridity in that it exhibits a tendency to be one-sided. Indeed dioecism may be described as a complete one-sided and alternative impotence that has arisen out of hermaphroditism. Intersexualism may occur in all grades or degrees as is shown by Goldschmidt (1916, 1917), Banta (1916, 1918), and the writer (1919) and is of course a widespread developmental process leading to a complete sex differentiation of individuals as contrasted with the differentiation of sex organs in a single individual. In respect to specialization of the individual as a whole intersexualism is a period of progressive variation.

In intersexuality various grades of maleness and femaleness may develop for individuals as wholes, or for particular sex organs as such. What is perhaps the best analysis of such phenomena in Mendelian terms (by Goldschmidt) recognizes that the assumed factors involved are themselves variable, that maleness and femaleness are properties of all cells, that the factors for sex are the same as factors for general growth, and that these are subject to much variation in relative potency.

There is therefore a decided analogy between the variations in the physiological condition of sex organs as revealed by their relative functioning in incompatibilities and those variations in the development of sex organs that are recognized under the term intersexualism.

Sex differentiation is hence widely variable in both its morphological and its physiological aspects.

4. Sex-determination and sex-differentiation in hermaphrodites are fundamentally a process of ontogenetic development, and may occur at various stages in ontogeny.

In hermaphrodites maleness and femaleness are both qualities possessed by all cells. Any nuclear organization or combination resulting after reduction division can become male or female according to whether the cell lineage leads through stamens or pistils. The obvious differentiation of the two sexes, morphological and physiological, may begin in the development of entire branches, or of flowers as wholes, or of stamens and pistils of the same flower, and is at first strictly a somatic differentiation of like diploid cells. The sexual nature of these sporophytic structures is however seen in the intimate part which they play in the production and function of the haploid sex generation.

A self-incompatible plant is itself the result of a compatible fertilization. Cytoplasmic and nuclear elements of an egg and a sperm fuse to form a zygote highly vigorous and of high sexual potentiality, yet its sex organs fail to function together. The elements which were compatible in the fertilization and in the life of the resulting zygote became incompatible during ontogeny. Yet the incompatibility does not arise simply because of the element of constitutional similarity involved in hermaphroditism, nor because of sex-differentiation as such, for a sister plant with the same parentage and ancestry may be highly self-compatible.

Sexuality is a cyclic recurring condition which makes possible the fusion of cells and nuclei and the pairing of chromosomes. The incompatibilities exhibited in processes of fertilization are due to physiological properties that are acquired during sex differentiation.

Whether the most successful fertilization depends on some element or degree of similarity, or on some degree of dissimilarity, or on a proper balance of the two, it is clear that the behaviour of incompatibilities both self and cross gives no proof that unlikeness in the sex organs favours the union of gametes, or that some element of similarity leads to incompatibility.

NEW YORK BOTANICAL GARDEN,

May 29, 1919.

EXPLANATION OF PLATES.

PLATE III.

All reproduced about twice natural size.

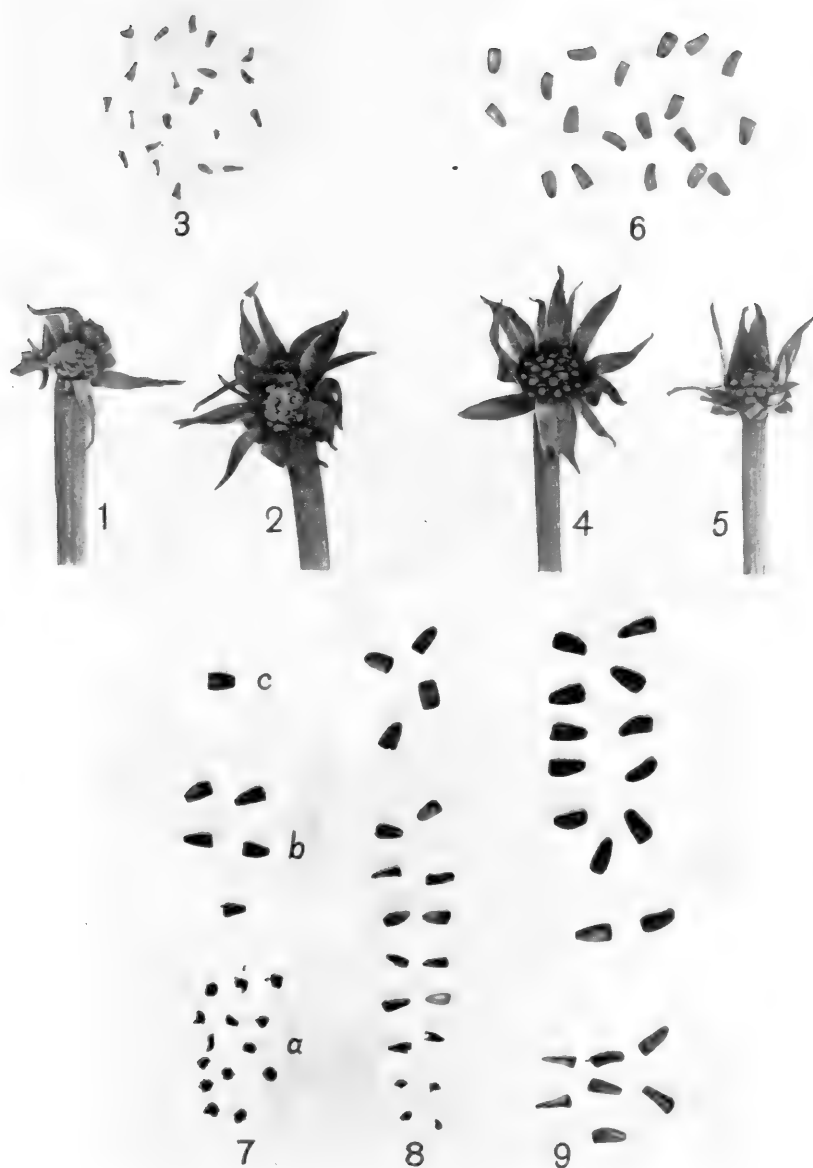
- Nos. 1 and 2 are heads, and 3 the rudimentary achenes of a third head all resulting from self-pollination of a completely self-incompatible plant of chicory; Nos. 4 and 5 are heads, and 3 the fully developed achenes of a third head resulting from a compatible cross-pollination of same plant on the same date.
- No. 7. Stages in development of achenes in a single head of a feebly self-compatible plant to self-pollination; at *a*, 14 merely rudimentary achenes; at *b*, 5 achenes of good size but containing no good embryos; at *c*, one seed with fully developed embryo.
- No. 8. Series of stages quite as in No. 7.
- No. 9. Achenes from a self-pollinated head of a plant strongly self-compatible. The greater number of achenes containing good embryos. No mere rudiments of achenes present.

PLATE IV.

View of chicory in experimental plot showing races of vigorous vegetative growth in the I_2 generation of self-fertilized descent. At the right, field label No. 26 marks the *Series 10-8-140*. To left of label 26 are four rows of two series of the family *12-11-*. To the extreme left may be seen bags on very tall plants of *Series 10-8-37*.

REFERENCES.

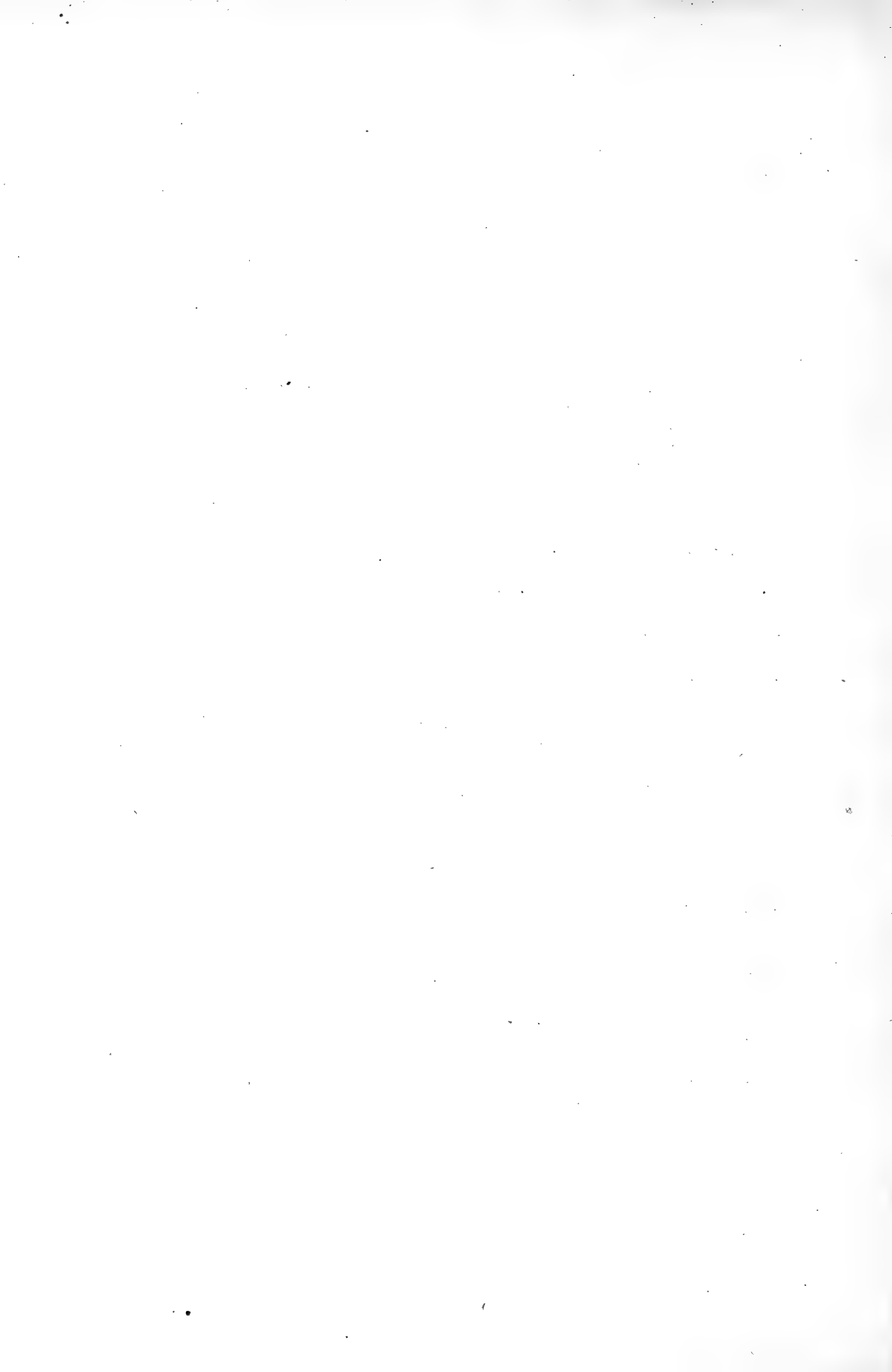
- BANTA, A. M. 1916. "Sex intergrades in a species of *crustacea*." *Proc. Nat. Acad. Sci.* Vol. II. pp. 578—583.
- . 1918. "Sex and sex intergrades in *Cladocera*." *Proc. Nat. Acad. Sci.* Vol. IV. pp. 373—379.
- BELLING, JOHN. 1918. "Lethal factors and sterility." *Journ. Heredity*, Vol. IX. pp. 161—165.
- CORRENS, C. 1912. "Selbststerilität und Individualstoffe." *Festsch. Med. Nat. Ges. z. 84. Versam. Deutsch. Naturf. u. Ärzte*, also same, 1913. *Biol. Centralb.* Vol. XXXIII. pp. 389—423.
- DARWIN, C. 1865. "On the sexual relations of the three forms of *Lythrum Salicaria*." *Proc. Linn. Soc. Bot.* Vol. VIII. pp. 169—196.
- . 1869. "On the character and hybrid-like nature of the offspring from illegitimate unions of dimorphic and trimorphic plants." *Proc. Linn. Soc. Bot.* Vol. X. pp. 393—437.
- . 1876. "Cross- and self-fertilization in the vegetable kingdom." Edition by John Murray.
- . 1877. "The different forms of flowers." Edition by John Murray.
- DETJEN, L. R. 1916. "Self-sterility in dewberries and blackberries." *Tech. Bull.* 11, N. Carolina Exp. Sta.
- EAST, E. M. 1915. "The phenomenon of self-sterility." *Am. Nat.* Vol. XLIV. pp. 77—87.
- . 1918. "Intercrosses between self-sterile plants." *Mem. Brooklyn Bot. Garden*, Vol. I. pp. 141—153.
- and PARK, J. B. 1917. "Studies on self-sterility. I. The behaviour of self-sterile plants." *Genetics*, Vol. II. pp. 505—609.







- . 1918. "Studies on self-sterility. II. Pollen-tube growth." *Genetics*, Vol. III. pp. 353—366.
- EWERT, RICHARD. 1909. "Neuere Untersuchungen über Parthenokarpie bei Obstbäumen und einigen anderen fruchttragenden Gewächsen." *Landwirt. Jahrbücher*, Vol. XXXVIII. pp. 767—839, Pl. XIV.
- GARDNER, V. R. 1913. "A preliminary report on the pollination of the sweet cherry." *Oregon Exp. Sta. Bull.* 116.
- GOLDSCHMIDT, RICHARD. 1916. "Experimental intersexuality and the sex-problem." *Am. Nat.* Vol. L. pp. 705—718.
- . 1917. "A further contribution to the theory of sex." *Journ. Exp. Zool.* Vol. XXII. pp. 593—611.
- GOODSPEED, T. H. 1915. "Parthenogenesis, parthenocarp and phenospermy in *Nicotiana*." *Univ. California Pub. Bot.* Vol. v. pp. 249—272.
- HEMSLEY, W. B. 1905. "*Nicotiana Forgetiana*." *Bot. Mag.* Vol. CXXXI. Tab. 8006.
- HERIBERT-NILSSON, N. 1916. "Populationanalysen und Erbliehkeitsversuche über die Selbststerilität, Selbstfertilität und Sterilität bei dem Roggen." *Zeit. Pflanzenzuchtung*, Vol. IV. pp. 1—44.
- JONES, D. F. 1918. "The effects of inbreeding and crossbreeding upon development." *Conn. Agric. Exp. Sta. Bull.* 207.
- KRAUS, E. J. 1915. "The self-sterility problem." *Journ. Heredity*, Vol. VI. pp. 549—557.
- and KRAYBILL, H. R. 1918. "Vegetation and reproduction with special reference to the tomato." *Oregon Exp. Sta. Bull.* 149.
- MORGAN, T. H. 1904. "Some further experiments on self-fertilization in *Ciona*." *Biol. Bull.* Vol. VIII. pp. 313—330.
- . 1910. "Cross- and self-fertilization in *Ciona intestinalis*." *Arch. Entwicklungsmech. Organ.* Vol. XXX². pp. 206—234.
- SHAMEL, A. D. 1918. "Why navel oranges are seedless." *Journ. Heredity*, Vol. IX. pp. 246—249.
- SIRKS, M. J. 1917. "Stérilité, auto-incompatibilité, et différenciation sexuelle physiologique." *Arch. Néerland. Sci. Exactes et Naturelles*, Sér. B, Tome III. pp. 205—234.
- STOUT, A. B. 1916. "Self- and cross-pollinations in *Cichorium Intybus* with reference to sterility." *Mem. N. Y. Bot. Gard.* Vol. VI. pp. 333—451, Pl. 30.
- . 1917. "Fertility in *Cichorium Intybus*: The sporadic appearance of self-fertile plants among the progeny of self-sterile plants." *Amer. Journ. Bot.* Vol. IV. pp. 375—395.
- . 1918 A. "Fertility in *Cichorium Intybus*: Self-compatibility and self-incompatibility among the offspring of self-fertile lines of descent." *Journ. Genetics*, Vol. VII. pp. 71—103, Pls. 4—6.
- . 1918 B. "Duplication and cohesion in the main axis in *Cichorium Intybus*." *Mem. Brooklyn Bot. Gard.* Vol. I. pp. 480—485, Pl. 12.
- . 1919. Intersexes in *Plantago Lanceolata*. *Bot. Gazette*, Vol. LXVIII. pp. 109—133, Pls. 12—13.
- SUTTON, IDA. 1918. "Report on tests of self-sterility in plums, cherries, and apples at the John Innes Horticultural Institution." *Journ. Genetics*, Vol. VII. pp. 281—300, Pl. 15.



METHODS OF DEGENERATION IN THE OSTRICH.

BY PROF. J. E. DUERDEN, M.Sc., PH.D.,

Professor of Zoology, Rhodes University College, Grahamstown; Officer-in-Charge, Ostrich Investigations, Grootfontein School of Agriculture, Middelburg, C. P., South Africa.

(With Plates V and VI, and 8 Text-figures.)

CONTENTS.

	PAGE
Introduction	131
Under-coverts : First Row ; Second and Third Rows . .	135
Upper-coverts	141
Remiges or Wing Quills	144
Leg Coverts	151
Bald Head Patch	153
Down	155
Digits of the Wing : Plumes on Third Finger	157
Fourth Outer Toe and Claw	162
Scutellation of Middle Toe	169
Degeneration and Adaptation	177
Ontogenetic and Phylogenetic Degeneration	180
Cause of Degeneration	184
Factorial Changes	187

THE Ratitae or running birds, as contrasted with the Carinatae or flying birds, have long been regarded as a degenerate group. A loss of structural parts has taken place in various directions, yet on somewhat similar lines throughout, pointing to some retrogressive influence common to all the representatives of the sub-class. The loss of effective barbicels, leaving the vane of the feather loose and open and incapable of offering resistance to the air, has rendered flight impossible¹. Probably as a result of the same degenerative tendency, other losses of plumage have taken place, as well as a total loss or reduction in the size of the

¹ The barbicels have almost disappeared in *Struthio* but are much better developed in *Rhea*. Chandler in his paper, "A study of the Structure of Feathers," gives illustrations of the two on Plate 13, *Univ. Calif. Publ. Zool.* Vol. XIII. 1916.

wings, the disappearance of a sternal keel and of elements from the shoulder girdle, and a reduction in the number of toes. As illustrations of one degenerative phase or another we need only recall the well-known retrogressive features associated with the recently extinct moas, and the still surviving ostrich, kiwi, rhea and various cassowaries.

Among the Ratitae the African ostrich, *Struthio*, affords distinctive evidence of some degenerative power at work in the small size of the wings and the unique reduction of the toes to two, as well as in the absence of plumage in various directions. A close study of the bird reveals other losses in less manifest directions, diverse stages having been reached in different individuals of the race.

It would therefore be expected that both on its own account and as a member of a retrogressive sub-class the ostrich would be peculiarly favourable for a study of the manner in which degeneration proceeds, as possibly throwing some light upon the nature of variation and the methods of evolution generally. An abundance of material is available among the thousands of domesticated birds on the ostrich farms of South Africa, supplemented by a recent importation of North African birds for crossing with the southern. From these it becomes possible to compare the course of degeneration over practically the whole continent, while embryological material enables the various ontogenetic phases to be followed from the earliest stage onwards. The results resolve themselves into a series of more or less separate studies in degeneration, though with certain considerations common to all. Special attention is given to the more manifest features associated with the plumage, the fore-wing and the toes, though these by no means exhaust the directions along which loss has taken, and is taking place. The facts disclosed will also be seen to have an important bearing upon the ostrich as a domesticated bird, constituting as it does an important farming industry for South Africa.

Though the plumage is the only part of the ostrich of importance from a farming and industrial point of view, we are probably justified in remarking that it is more scantily provided with feathers than any other living bird. The under surface of the wings (Fig. 1), the lateral part of the body from the axillary region to the tail, as well as the entire surface of the legs, are practically devoid of feathers in the adult (Pl. VI, fig. 4); also a large apterium occurs over the sternal callosity in front, and another over the pubic thickening behind; in addition, the undercovering of down has all but disappeared from the race, while in the northern bird the top of the head is bald (Fig. 5, p. 153). Ordinarily the over-

lapping of the feathers covers the naked areas, with the exception of the legs; but on hot days, when all the feathers stand on end and the wings are out-stretched, the limited extent of the feathered areas (pterylae) becomes a conspicuous feature. On the other hand, the valuable wing plumes (remiges), for which the ostrich is prized, are almost unique among birds in their high number, as many as 44 having been counted, though the average is about 36¹. It will be shown that the marked absence of plumage in so many directions is largely a result of degenerative losses, for survivors of a more heavily plumaged ancestral state are yet to be found.

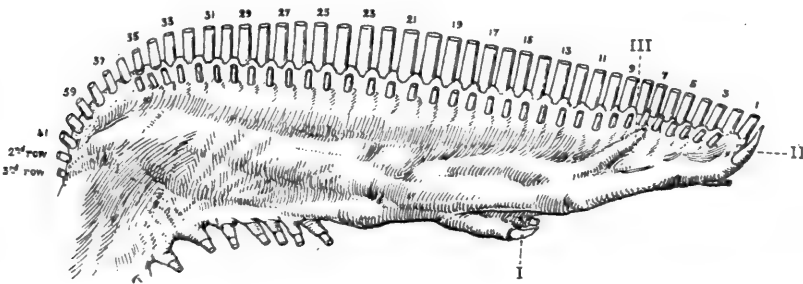


Fig. 1. Under surface of wing with plumes clipped off showing absence of feathers, except the single incomplete row of under-coverts. The marginal row consists of 41 remiges and the last plume of the first and the second row of upper-coverts. The row of under-coverts has one feather wanting at the beginning of the row and eight at the elbow end. (I) claw on first finger or, bastard-wing; (II) claw on second finger; (III) outline of third finger partly showing through the skin.

It is deemed to be evidence of degeneration if plumes or other structural parts are absent from positions where by comparison with other birds they would be expected to occur, more particularly if confirmatory proof is forthcoming from their presence as survivals in other ostriches. An ordinal succession of losses is presumed where all intermediate stages between the extremes can be procured, the retrogressive process having proceeded at different rates in different individuals, the various stages representing successive steps in the retrogressive evolution of the race. An attempt is made to interpret the changes in terms of the factorial hypothesis², though on account of the slow breeding nature of the

¹ Beebe (9th Ann. Rep. N. Y. Zool. Soc. 1905) states that the Albatross has 40 to 50 remiges, Rhea 28, Emeu and Apteryx 17, and Archaeopteryx 16.

While the paper was in preparation there appeared an article by Prof. T. H. Morgan, "Evolution by Mutation" (*The Scientific Monthly*, Vol. VII. No. 1, 1918). In this the author briefly summarizes what mutation has to offer in the way of a contribution to evolution, a timely exposition of which free use has been made.

ostrich the experimental confirmation of the issues involved is scarcely possible. About three years intervene between the beginning of one generation and the next; moreover all the birds are of mixed ancestry, sufficient time having not yet elapsed to build up strains which are "pure" as regards any one of the variable characters.

While still a matter on which geneticists are by no means agreed, and in the absence of experimental proof, it is held that the gradual diminution in size of a structure in a degenerative phase is most feasibly explained as evidence of a slow degradation on the part of the germinal factors concerned¹. The weakening is probably to be interpreted as the result of successive factorial losses, perhaps following upon fractionation, the expressive power of each loss being so small that the appearance of continuity is preserved. The "multiple allelomorphs" of Prof. Morgan indicate that a single unit factor may exist in a great number of grades, while the "multiple modifying factors" suggest that a visible character may be modified in the finest gradations by alterations in different parts of the germinal apparatus. These would appear to render unnecessary an appeal to a direct change in a factor itself. Prof. W. Bateson in his Presidential Address before the British Association in Australia in 1914 is however "satisfied that they may occasionally undergo a quantitative disintegration, with the consequence that varieties are produced intermediate between the integral varieties from which they were derived."

Where a retrogressive mutation shows Mendelian dominance the presence of a factorial influence of an inhibitory nature would seem to be justified; or it may be that a factor for a somatic loss is dominant over one for its presence. Where Mendelian recessiveness is obviously not involved, and having regard to the general retrogression in progress, the disappearance of a structure is deemed to correspond with factorial loss, or possibly of degradation to such a degree that it is incapable of gaining somatic expression.

Reference is sometimes made to the supposed greater variability of domesticated animals as compared with wild animals. The conditions in the ostrich are clear on this point. Though all the examples studied

¹ As modifying a simple attitude of this kind Prof. Morgan (*l.c.* p. 50) writes: "The hypothetical situation is, however, uncertain in a high degree the moment one realizes that changes up and down of a character appear more often brought about now by one, now by another gene, rather than of the principal one, i.e. the one on which, owing to a mutation, especial attention is focussed." And again, referring to palaeontologists as advocates of orthogenesis or continuous variation with a definite trend: "They overlook the fact that to-day there is experimental evidence demonstrating that variations as small even as those they record have been shown to rest on mutational changes."

are domesticated birds the change to this state from the wild one is so recent that it is impossible to conceive that retrogression of such a deep-seated nature as here described could have been instituted in so short a time, at most not more than fifty years; moreover, the imported northern birds were caught as chicks from the nests of wild birds, and display similar stages to those of the domesticated Cape bird.

UNDER-COVERTS.

First Row. In nearly all ostriches the under surface of the wing is naked except for the presence of a single row of under-coverts, and even this is rarely complete. The coverts alternate with the row of wing-quills, and also with the last plume of the first, second and third row of upper-coverts which, along with the former, constitute the post-axial, marginal row of plumes (Fig. 1). Only in two birds out of hundreds examined however has the full alternation of under-coverts been observed, and often as many as nine or ten are missing. Actual counting of twenty-five birds gave an average of 30.2 coverts, which is six less than the average of the remiges, and one, two, or three more should be present for alternation with the last member of the one or more rows of upper-coverts completing the marginal row. The absent members form a regular ordinal succession, beginning at the elbow end of the row, and all numerical stages from one to ten can be procured, though the greater number of birds have only six or seven missing. The remiges constitute a convenient standard from which the degree of loss of the coverts can be determined. They have a fluctuating variation of their own with which that of the coverts is correlated, but the latter have also an independent variation.

While usually the losses represent entire plumes, not infrequently one or two vestigial feathers are seen beyond the last ordinary one. These are sometimes merely diminutive entire feathers, but often they are small and imperfectly formed, the shaft especially being wanting. The stages of degeneration which they represent are similar to those more frequently found constituting the degenerative down on the wings and tail to be described later. Here all stages in the reduction in size of the complete feather are to be seen; then others in which the shaft breaks up into two or three imperfect parts and ultimately disappears, and only a tuft of barbs bearing barbules results; afterwards the barbules disappear, and, lastly, one or two hair-like barbs only remain (Fig. 2). These degenerate feathers continue to appear in the individual with the

regular succession of the plumages—chick, juvenal and adult. They are also found on chicks at hatching or even before hatching, showing that the particular stages reached are germinal and not ontogenetic productions.

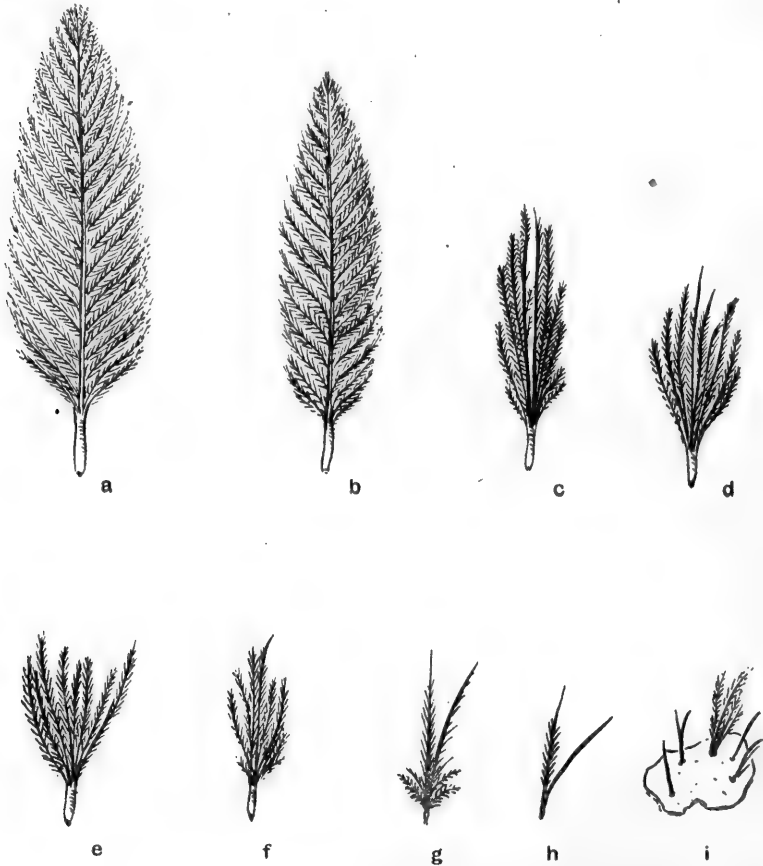


Fig. 2. Stages in degeneration of a feather. The feather first undergoes a gradual reduction in size; the central shaft then breaks up into two or three parts, as in *c* and *d*; the imperfect shafts disappear and a simple tuft of barbs with barbules is formed, *e*; these undergo a gradual reduction and loss of barbules until only one or two hair-like barbs remain, *f* to *i*.

Some birds afford evidence that a loss of coverts is beginning at the distal end of the row also. Usually no under-covert alternates with the first and second remiges (Fig. 1), though such would normally be expected. Occasionally however two, three, or even four alternations are

missing, or are represented by small, degenerate feathers on the way to disappearance. Hence while in most ostriches the under-coverts show retrogression only at the proximal or elbow end of the row, in some few a loss is beginning at the distal or free end also.

From the details submitted it is apparent that the single row of under-coverts in the ostrich is in process of gradual reduction, mostly at the elbow end of the row, but to a less degree at the distal end also. The alternative would be to regard the conditions encountered as representing the fixed normal fluctuations for the row; but the occurrence of vestigial feathers, and other facts to be presented, show that losses are actually taking place, and leave no doubt that retrogression is in progress in many directions. Even if the losses are not actually taking place at the present time they must have occurred at some period during the past history of the ostrich, and in the ordinal manner which they now display. The number of birds available renders it possible to trace all the stages from the rare complete row to one where as many as ten have disappeared, indicating that the process has not proceeded uniformly in all the representatives of the race. In most cases the absences involve complete feathers, but the occasional presence of the diminutive and vestigial members serves to prove that each loss has been brought about in a slow continuous fashion, according to a definite sequence.

Second and Third Rows. While by far the majority of ostriches have only the single, incomplete row of under-coverts, birds are occasionally found which bear feathers belonging to a second, and even to a third row. These additional feathers are more likely to be found on southern than on northern birds. In one Cape cock as many as thirteen alternating smaller plumes constitute a second row, and in front of these are four other plumes alternating with them, and manifestly members of a third row. They tend to give to the wing an appearance of a completely covered under-surface, altogether different from the usually naked effect. In other ostriches detached feathers of the second and third rows may be present at any part of the row, or several may appear in regular succession, the middle members of the row being more likely to occur than those towards the end. Only on one farmer's strain however have the nearly complete second and third rows been found, and they re-appear in the progeny, in more or less the same numbers. The feathers are nearly always fully formed and, like the first three rows of upper-coverts, are successively smaller from the first to the third row; occasionally, however, vestigial examples occur, representing degenerative stages of individual plumes.

The only feasible explanation which can be advanced for the rare presence of second and third row under-coverts is that the ancestors of the ostrich were, like modern flying birds, provided with a covering of feathers to the under or inner surface of the wing, though in nearly all cases this is now naked except for the single row of under-coverts, itself a disappearing quantity.

We appear to be justified in assuming that the loss of plumes from under the wing has been going on continuously in the ostrich race for a long period, but at a different rate in different individuals. In by far the majority of birds all have disappeared with the exception of the first row of coverts. The germinal factors for a regular succession in the second row still persist in one strain, and for a less number of the third row, while in other strains the factors for odd members only remain. No reasonable doubt can be entertained that the stages still surviving represent in a general way the ordinal succession according to which the factorial losses have proceeded and are still proceeding for the race as a whole, the factors for single plumes from one end of a row first, and then from both ends. The vestigial feathers frequently found next the ordinary ones reveal that the losses are not effected as complete plumes, but that each passes through a definite series of degenerative stages before its final disappearance. The loss proceeds by a gradual reduction in size, accompanied in the end by a breaking up and loss of the constituent parts.

An individual ostrich plume is made up of a number of constituent parts—quill, shaft, barbs and barbules. The minute structural details of all these vary greatly in different strains, the differences determining the commercial value of the plume, and therefore calling for intensive study on the part of the ostrich breeder. Selection in breeding, now extensively carried on, has fully established the fact that the diversities behave as independent characters, and must therefore have some separate factorial representation in the germ plasm. As a consequence the factorial constitution of even a single ostrich plume must be highly complex. A moment's reflection however suffices to show that the complexity generally acts in a unified manner. Thus a feather appears and develops in its entirety, and also responds as a whole to changing nutritive conditions, as if some common influence were controlling its component factors. The same cause it must be which determines the relative sizes of the plumes in a row where, as in the case of the remiges, the middle members are the largest, and a gradual reduction takes place towards each end. In the case of the diminutive feathers which represent the

beginning of degeneration, it is the feather as a whole which becomes reduced in size, all its structural parts becoming smaller in due proportion. Manifestly in this case the common controlling influence is undergoing some slow degradation, and all the constituent factors are correlated with it; unless, with Morgan, we think of the retrogressive changes as being due to other genes, multiple modifying factors, rather than the principal one.

In the later stages of degeneration where, in addition to continued diminution, actual loss of the structural parts of the feather occurs, it is clear that the separate factors of the feather are losing their potency, and in the end cease to gain somatic expression. The retrogression for these proceeds according to the following sequence: *first*, a weakening and loss of the factors concerned with the shaft; *second*, of those representing the barbules; and *third*, of those responsible for the barbs. Thus two influences seem to be at work: one resulting in a weakening of the factor or factors which control the feather as a whole, and the other, and later one, in a weakening and ultimate loss of those which determine the structural details. Where retrogression is in progress it by no means follows however that a factor is lost to the germ plasm as soon as it fails in somatic expression. Evidence from what seem to be "reversions" supports the idea that the factors may continue in the germ plasm after losing their ordinary power of somatic manifestation, and presumably they continue their course of degradation until, as it were, they finally atrophy.

Whatever the degenerative influences within or acting upon the germ plasm may be, they proceed slowly and continuously for the race as a whole, but with some variability in the rate of action in different strains. All intermediate conditions between the extreme stages are easily forthcoming; there is no suggestion of any abruptness or break between one and the other. If, in the case of the diminution in size of the individual feather, we hold that the changes are the expression of a single controlling factor the latter must be undergoing a gradual reduction in power, whereas if the loss of a multitude of constituent factors is concerned the expression of their individual loss is so small as still to leave the impression of continuity; even if modifying inhibitory factors are invoked their action must be effected in the same slow, sequential manner. The factorial view adopted has obviously no pragmatical bearing upon the results of degeneration, as contributory to the evolution of the ostrich; but the hard fact of continued, rectigrade mutations has to be faced by Mendelism.

The degenerative influence, having resulted in the complete or partial loss of a plume, then begins to attack the next one or two in the ordinal sequence of the row, and thus a regular, successional reduction is maintained for the row as a whole, though not more than one or two members are in a retrogressive phase at the same period. This appears to be the limit of the degree of retrogressive activity for the time being as regards any row, an independence for each end being maintained, and also for the different rows. The succession of losses is never irregular or haphazard, as would be the case if a plume disappeared in the course of the row instead of from one or other of the extremities.

That in the end the factors become completely lost to the germ plasm may be inferred from the fact that missing members are wanting from the earliest appearance of the plumage, and only re-appear in the naked part of the row in certain strains in which their degradation is not yet completed. Examination of embryos when first showing feathers reveals similar absences, as do chicks when first hatched and the adults later. The degenerative losses are therefore not effected during ontogeny, but during the complex mitotic germinal changes which intervene between one generation and the next. Dr C. B. Davenport writes¹: "We are ignorant of the specific nature of the machinery that determines phylogenetic variations, but we have reason to think that it is located in the germ plasm and that the karyokinetic phenomena, especially the movements of chromosomes at and around the time of fertilization, have a great deal to do with such phylogenetic change." Prof. Bateson in his Presidential Address in Australia also remarks: "It is to be inferred that these fractional degradations are the consequence of irregularities in segregation." The phylogenetic changes which Bateson, Davenport and others usually have in mind in this connection are the larger, sudden, detached changes regarded as discontinuous variations (Bateson) or mutations (de Vries). Their fortuitous isolated character can be readily understood if we conceive of them as resulting from irregularities during mitotic division and reconstruction; but it is manifest that the continued, determinate phylogenetic changes exhibited by the ostrich plume could not be a product of mitotic irregularities. The facts call for a regular succession of germinal changes of a uniform character in the same direction, continued generation after generation; they must be sequential and cumulative, and the results would admit of accentuation by selection.

Several writers have drawn attention to the fact that the occurrence

¹ *Amer. Nat.* Vol. L. August, 1916.

of a continuous series of variations from one extreme stage to another does not necessarily represent a successional germinal series, nor indicate an evolutionary trend in any determinate direction. In his studies on *Drosophila* Prof. Morgan, for example, shows that a continuous series of intermediate eye-colours between the two extremes, deep brown sepia and pure white, could be picked out; also a graded series with perfectly formed wings at one extreme and no wings at the other. But the experimental evidence reveals that the various stages are produced as separate mutations, independently of one another, not as a sequential series, and that large steps occur as well as small ones. Detached changes of this kind are not steps in evolution unless they confer selection value upon their possessors, but are examples of the small, fortuitous germinal variations probably always to be found in a mixed assemblage of any species. In the ostrich, by contrast, everything indicates that the variations are successional in their origin, that all have a retrogressive trend, and are a part of the evolutionary process common to the race; the different individuals represent the different evolutionary stages of the race. From the evidence here presented no one can doubt, for example, that we have a definite sequential trend in the loss of the toes from the foot; also in the loss of the coverts from the wing, the one or two diminutive or vestigial feathers at the end of a retrogressive row being taken to represent the magnitude of the losses in progress for the time being. It is claimed that the ostrich is in a retrogressive evolutionary phase at the present time in various directions, and that the different stages represented by the different members of the race afford us the means by which the methods followed can be determined.

UPPER-COVERTS.

The upper-coverts are black in the cock and grey in the hen and, like the under-coverts, regularly alternate with the wing-quills (Figs. 3 and 4, and Pl. V, fig. 1). Usually only the first two rows are clipped as part of the feather crop. The complete number of rows however varies in different individuals. In some only two or three are clearly defined (Fig. 4), but five or six in others, or even more if the counting be continued to the short rows near the pre-axial border of the wing (Fig. 3). As regards the number of plumes to each row, the first one is found to vary in direct correlation with the wing-quills and rarely exhibits any independent reduction. The first member alternates with the first and second wing-quills; the last one extends beyond the last wing-quill and

is situated in the marginal row. It is evident therefore that the degenerative influence has not yet reached the first row of coverts, except in so far as it varies with the remiges. These latter will be seen to vary from 33 to 44, and as the first row of upper-coverts is always in numerical agreement, some correlating influence between the remiges and coverts must necessarily be present.

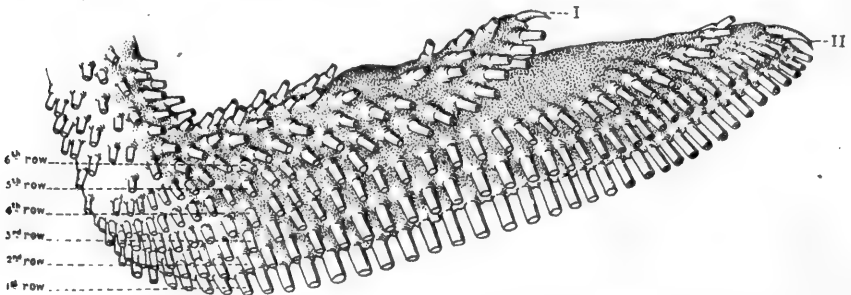


Fig. 3. Outer surface of wing showing more clearly the arrangement of the rows of feathers. South African ostrich with two complete rows of upper-coverts and several shorter rows, all continued round the elbow.

The second row of upper-coverts on the other hand is in a marked degenerative phase, the losses taking place for the most part at its distal end, in contrast to the proximal end of the first row of under-coverts. The full normal number necessary for complete alternation with the first row of coverts is not often present (Fig. 3); sometimes six or seven, or even as many as ten, are missing, but all intermediate numbers are to be found (Fig. 4). Occasionally one or two of the feathers in front of the

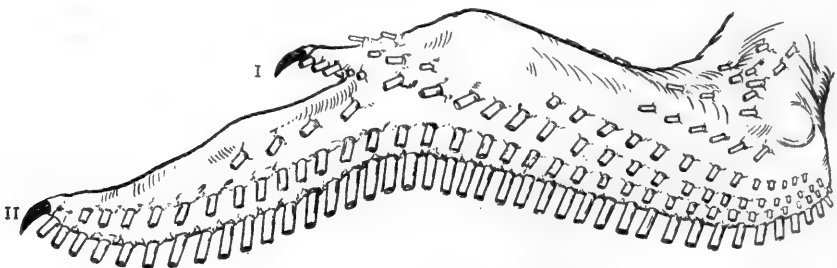


Fig. 4. Wing of North African ostrich with extreme reduction of feathers (cf. Fig. 3). The first row of upper-coverts with only 35 plumes has five less than the marginal row, and the second row is still further reduced, as well as the number of smaller rows. The wing as a whole is narrower than that from which Fig. 3 was drawn, and the plumes are not continued round the elbow.

first ordinary one are diminutive or vestigial, as if undergoing degeneration in the same manner as described for the under-coverts. The third row of upper-coverts is nearly complete in some southern birds, but rarely in northern; along with the succeeding rows it often shows a rapid shortening from the proximal end forwards.

The region of the elbow, between the plumes of the fore-wing and those of the upper-arm, is also one in which plumage reduction is in progress. In some southern birds no sharp line of separation can be drawn between the two, the feather rows of the fore-wing passing uninterruptedly into those of the upper-arm (Fig. 3); but in most northern birds an irregular naked area occurs, and the proximal limits of the different rows are then well defined (Fig. 4). In these cases the second and third rows of upper-coverts are not continued upwards so far as to end in the marginal row, but terminate above it. Thus the rows of upper-coverts may be undergoing reduction at the elbow end of the row, though the chief loss hitherto is from the distal end.

From the above it is clear that, if the reduction of the upper-coverts continues along the lines indicated, the outer surface of the fore-wing will in time become as naked as is the present under surface, the manner and order of succession of the losses being much the same in both cases. In each a loss in the number of rows takes place, as well as of the individual feathers from each row. A study of the one is supplementary to a study of the other, and the results are confirmatory, though at the present time the two surfaces exhibit very different degrees of degeneration.

In correlation with the number of rows of upper-coverts a marked structural difference is displayed in the width of the fore-wing. In some northern birds only the remiges and first-row coverts extend the entire length of the wing, the other rows being reduced in number and scarcely passing beyond the proximal half, while in some of the best Cape birds two or three rows of coverts may be practically complete, followed by a number of other shorter rows (cf. Figs. 3 and 4). Taking the more extreme cases it is found that where the rows are complete and numerous the fore-wing structurally is proportionately broad, while where the number is small it is distinctly narrower. Were the measurements of a large number of wings to be taken and also the number of feathers borne by them, there is no question that a distinct correlation ratio would be disclosed. A marked degree of independence undoubtedly characterises most of the directions along which degeneration is taking place, but it is manifest that certain correlations may also appear. The words of

Prof. T. H. Morgan (*l.c.* p. 50) seem pertinent: "Moreover, the fact that recent work has made clear that genes generally have more than a single effect on the organization opens wide the door of suspicion, for the observed morphological progress might be a by-product of influences that have other and important, though unseen, effects."

A structural narrowing might be anticipated where the number of rows of coverts is in course of reduction if the wing of the ostrich as a whole be undergoing degeneration, and from comparison with other *Ratitae* a change of this nature seems likely. A shortening of the wing also may be looked for in correlation with a reduction in the number of remiges and coverts to the rows as a whole, but no relationship of the kind is yet manifest, even though the former vary from 44 to 33. The matter would probably assume a different aspect however if the remiges commenced to undergo reduction at the distal or free end of the row, instead of being restricted to the proximal or elbow end as at present.

REMIGES OR WING QUILLS.

Closely associated with the coverts are the remiges or wing-quills. They comprise the familiar white plumes of the ostrich and a few parti-coloured feathers from each end of the row, the so-called *byocks* or *fancies*. They are the most valuable of all the ostrich plumes, and from a farming and breeding point of view call for more consideration than all the other kinds combined. Quality in them counts for far more than quantity. The latter however has an importance, seeing that to a large extent the number of remiges also determines the number of coverts alternating with them.

The wing plumes have been counted on large numbers of both northern and southern birds and the results published¹. It is shown that on the wing of the northern bird they vary from 33 to 39, while on the southern bird they fluctuate from 33 to 42, the latter number since increased to 44. In a mixed assemblage, uninfluenced by selection, both northern and southern countings form an approximately normal curve of fluctuating variation, with the mode at 36 and the arithmetical mean about 36.5. Before the present investigations only one southern bird with the high number 42 was known, while the numbers 41 and 40 were extremely rare. These few scarcely influence the average, and for

¹ "Breeding Experiments with North African and South African Ostriches; IV. Increasing the Number of Plumes; Degeneration and Restoration," *Dept. of Agric., Union of South Africa*, Bull. No. vii. 1918.

ordinary purposes it may be held that the northern and southern birds have the same average of plumes. By selective breeding numbers of chicks bearing more than 40 plumes are now being reared.

Whether degeneration is in progress among the remiges of the ostrich at the present time can only be indirectly established, for no standard exists with which the number in the row can be compared. In the case of the coverts any loss is at once apparent, since the normal alternations with the remiges are wanting. The wide variations from 44 to 33 might possibly be regarded as the normal fluctuation of the mixed race of ostriches, and it will be shown that each plume has distinct factorial representation in the germ plasm. As in fluctuating series generally few individuals are represented at the two extremes, but more are collected about the middle. To establish degeneration statistically it would be necessary to show that the average for the whole race is undergoing retrogression, but this is manifestly impossible.

Comparison of the wings of high and low numbered ostriches reveals that losses are effected at the elbow end of the row. In high numbered birds the remiges extend round and beyond the elbow projection, and pass for a short distance along the upper arm, an unusual relationship in birds generally; but in low numbered they scarcely reach to the end of the elbow, and a wide interval separates them and the first rows of upper-coverts from the rows of the upper-arm, rendering counting much easier (cf. Figs. 3 and 4). Occasionally one or two diminutive or vestigial feathers occur at the termination of the row, similar to those found at the ends of the covert rows, and these may be regarded as satisfactory testimony that reduction is actually in progress, particularly when taken in conjunction with the facts of degeneration generally. The process must however be very slow and fairly uniform for the race as a whole, free from anything fortuitous, seeing that both the northern and southern birds bear the same average. It may be held that the ancestral ostrich would have a fixed number of wing-quills at the beginning, and that any later departures from this would be in the direction of reduction, not of increase; hence we may regard all losses below 44 as so many losses of individual plumes and as retrogressive mutations. The later embryos show the same variations as the adults, a proof that the germinal factors concerned with the lost plumes have disappeared from the germ plasm, following the same ordinal sequence from the proximal end outwards.

None of the ostriches in Africa are yet germinally pure so far as concerns the number of wing-quills. The crosses show them to be

heterozygous, as would be expected from the mixed breeding hitherto carried on and the wide variations represented, and the high numbers are dominant over the low. Experiments to establish germinal purity are however in progress. It is hoped to demonstrate that each successional number of wing plumes represents a germinally pure line, that is to say, that each plume has distinct factorial representation in the germ plasm. Without doubt the variations already noted among the coverts are also capable of extraction as numerically pure lines, but to establish it experimentally would be a prolonged undertaking. If therefore the factorial individuality of the variations in the wing plumes can be proved it may be admitted for the rows of coverts also and for the feathers generally.

The matter of genetic purity of the remiges has also an important industrial application, for if established it becomes possible to provide the ostrich farmer with a bird giving at least the high average of 44 plumes to the wing in place of the degenerative average of 36 with which he now farms, the coverts being increased in like proportion. As indicating the likelihood of ultimate purity the series given below in Tables I—III have been selected from a number of others of a like character.

TABLE I.

<i>Breeders :</i>				Right Wing	Left Wing
South African cock, No. 229 ¹				41	43
Cross hen, No. 233				41	43
<i>Chicks :</i>					
No. 1 ¹	40	41
No. 2	41	39
No. 3	44	44
No. 4	40	40
No. 5	39	40
No. 6	43	42
No. 7	39	39
No. 8	43	42
No. 9	43	43

The above represents the best effort at present to establish a numerically "pure strain" of ostriches from the highest numbered birds available, and it must be admitted that the results are encouraging. In both cases the parents have a mixed ancestry, having been reared before the question of plumage number was under consideration. The parental details of the South African cock are not available, while the cross-bred hen is from a Cape cock having 42 plumes to the wing mated

¹ The high numerals are those given the adult birds in the ostrich register, the low successional numbers apply to young chicks which usually do not receive their register number until about six months old.

with a northern hen bearing only the average numbers 36 and 37. No retrogression to these low, grand-parental numbers occurs, so the influence of selection is already manifest, the high numbers tending to dominate the low, and the series shows an approximation to purity as regards high numbers. No doubt can be entertained of the possibility of reaching a still higher degree of purity by continued selective breeding. It is submitted that the results, along with those in the two following series, are sufficiently approximate to be already accepted as evidence that each plume has a separate factorial representation in the germ plasm, and that the variations from 33 to 44 are not environmental. The natural ostrich may well serve as an illustration of the principle embodied in the following remark by Prof. H. S. Jennings¹: "All thorough work has led directly to this result: that any species or kind of organism is made up of a very great number of diverse stocks, differing from each other in minute particulars, but the diversities inherited from generation to generation."

However high the degree of germinal purity which may be attained by continued selective breeding in the ostrich it is however problematical whether a uniform number of plumes will ever be procured in the progeny. The slight variations between the right and left wings shown in Tables I to III are an indication that departures of one or two plumes from the expected number will probably always occur. The fact that the plumage of the ostrich is in a degenerative phase, and presumably the genetic factors also, has got to be reckoned with. On many of the above chicks, as also among those of the two series below, one or two diminutive or vestigial feathers are to be found at the elbow end of the row of remiges, showing that retrogression is in process, and sometimes a vestigial feather will occur on one wing of a bird and not on the other. As regards these one or two degenerate feathers therefore the genetic factors concerned may be held to be weakening and variable by one means or another, and in this state it is evident that they may or may not gain somatic expression, even on one wing as compared with the other. If this be the correct interpretation it is to be expected that, however purely bred, the same degree of variation will be found among the progeny as between the right and left wings of the individual. If the same zygote can give rise to a marked bilateral variation different zygotes from the same parents may also be expected to produce a like variability in the progeny.

¹ Jennings, H. S., "Observed changes in Heredity Characters in relation to Evolution," *Jour. Wash. Acad. Sci.* Vol. VII. 1917.

As will be seen later in connection with the other paired structures undergoing degeneration, particularly the claw on the fourth toe and the scales on the third, a similar variation often appears between the two sides, and would also seem to find its explanation in the fact that the genetic factors concerned are in a changeable condition. Where factors are undergoing retrogression, whatever their nature, a stage will naturally be reached so near the margin of somatic expression or non-expression that uncertainty will occur as to whether they gain expression or not, varying conditions in the soma perhaps having some influence. The result will be particularly manifest in the case of normally paired structures, if they should appear on one side of the body and not on the other. Where structural features are in a degenerative phase slight variations in the progeny from the same parents, as well as between the right and left sides of an individual, will therefore not necessarily be evidence of germinal impurity, but an indication of factorial weakness; neither will they be testimony to environmental influences, such as manifest themselves among the individuals of a pure genotypic line, for normally any numerical fluctuations are germinal¹.

The conclusion reached has obviously an important bearing upon the general question of germinal purity and its manifestation in the soma. Only when the genetic factors are fixed and stable can we hope for somatic purity, or be in a position to extract genotypic lines altogether pure in their characteristics. Contrariwise, where genotypic purity is attained it may be deemed to be evidence that the factors are in a settled state, as in Johannsen's well-known experiments with beans and those of Jennings with *Paramoecia*, where the characteristics for each line were found to be constant and only environmental variations appeared. One advantage in connection with genetic studies on the plumes of the ostrich is that the differences are numerical and therefore particularly concrete, and the numbers do not vary with external influences, nor any changes introduced in the course of the life-time. Moreover, by dealing with concrete numbers none of the fluctuating

¹ Dr A. F. Shull (*Amer. Nat.* Vol. LI. 1917, p. 365) refers to the fact that Hyde has recently reported a case in *Drosophila* in which the two *X* chromosomes appear to have remained undivided, going to opposite daughter cells, and resulting in the production of right and left eyes of different sex-linked colours. While one can conceive of occasional cases of the failure of chromosomes to divide during mitosis, thereby giving rise to asymmetry, it can scarcely be held for the ostrich where bilateral irregularity is so frequent. Factorial degradation to near the margin of expression appears a more likely interpretation, especially when considered in conjunction with the general facts of degeneration presented by the ostrich.

variations which Johannsen and Jennings encountered will occur. From the variations between one chick and another not much that is decisive can be gained where the parents are admittedly of mixed ancestry, but when we find variations between one side and the other in so many features it becomes clear that we are encountering some factorial irregularity.

The diminutive and vestigial feathers are somatic evidence of factorial degradation, and it is submitted that the variations as between one wing and the other of the same bird reveal that the factors are approaching the limit of their power of expressibility, and that in this state they may or may not act with strict regularity for different individuals or for the two sides of bilateral structures. It seems trite to repeat that, however pure the breeding may be, uniformity of characters can not be expected if the factors themselves are in a changing or very weakened phase, but only when they are fixed and stable¹.

TABLE II.

<i>Breeders :</i>				Right Wing	Left Wing
North African cock, No. 284				36	36
North African hen, No. 87				37	37
<i>Chicks :</i>					
No. 1	37	37
No. 2	36	38
No. 3	36	37
No. 4	39	38
No. 5	36	35
No. 6	36	37
No. 7	35	36
No. 8	39	39
No. 9	37	37

Table II represents the number of plumes on a hatching of North African chicks of which the parents have the average number of plumes. Both the cock and the hen are imported northern birds of mixed ancestry and the results show an approximation to the parental numbers, though with slight variations above and below, but by no means approaching the wide extremes characteristic of the race. The numbers are wholly different from those of the former series, except that the highest, 39, in the one is the lowest in the other.

¹ In his lecture, "The rôle of Selection in Evolution," before the Washington Academy of Sciences in 1917, Prof. W. E. Castle remarks that the pure line principle "does not fit in with the observed facts as regards the effects of selection in the majority of the domesticated animals and cultivated plants, nor even with the behaviour of certain characters in self-fertilised plants and asexually propagated animals." He refers to the genetic variations encountered by Jennings with *Drosophila*, his own with guinea-pigs, rabbits and rats, and that of various workers with asexually reproducing plants.

Here, as in Table I, we have variation as regards the number of plumes grown by each chick, probably indicating lack of germinal purity in the parents for the most part; and we have also variations of one or two plumes as between the two wings of the individual, pointing to a lack of factorial stability in connection with the one or two end plumes, and perhaps also influencing the individual variation resulting from the mixed ancestry.

TABLE III.

<i>Breeders:</i>					Right Wing	Left Wing
North African cock, No. 84					34	35
North African hen, No. 116					33	33
<i>Chicks:</i>						
No. 1	36	36
No. 2	35	35
No. 3	34	34
No. 4	34	34
No. 5	34	35
No. 6	34	34
No. 7	33	35
No. 8	34	34
No. 9	35	34
No. 10	34	34

In Table III both the cock and hen represent the lowest plumage numbers available among the breeding birds, and were selected to determine how far the low numbers would breed true; also if it would be possible to reduce the plumes beyond the minimum yet known, namely, 33. It must be admitted that the ten chicks show a remarkable uniformity considering the mixed parentage, the extreme variations, 33 to 36, representing a difference of only three plumes, while in the second series the difference, 35 to 39, is four, and in the first series, 39 to 44, as many as five. Many of the chicks show vestigial feathers at the elbow end of the row of remiges and those of the coverts, while in one as many as four under-coverts are absent from the distal end of the row, a most unusual number.

If the terminal remiges are in a more or less degenerative phase in all ostriches, as is here maintained, we may expect that retrogression will be hastened by breeding together ostriches in which the change is most in evidence. Any loss achieved in this way would be most manifest in birds producing the minimum number of plumes, as the present limit of variation would be still further lowered. By selecting the lowest numbered birds available it should be possible still further to reduce the number, particularly if in-breeding were carried out. It would naturally constitute direct experimental evidence of degeneration and the influence of selection if such a reduction could be effected. It

is hoped that it may be possible to attempt this, but with such a slow breeding animal the number of years required is not an encouraging contemplation. The building-up of a pure strain of the highest numbered birds has a high industrial bearing, but the production of low-numbered birds is a retrogressive step.

With the remiges in their present degenerative phase, dropping out in ordinal succession one after another from the elbow end of the row, there should be no limit to which the losses may proceed, given the required time; they should continue until all the wing-quills have disappeared, as is already the case with the second and higher rows of under-coverts. It is otherwise however with any addition to the upper limit. No degree of selection can increase this if the factors were never there ancestrally, or if they have been altogether lost to the race. As 43 plumes was the highest number previously encountered it was with some surprise that chicks with 44 were found in the first series. A margin of one or two is however to be expected in view of the considerations already given as regards factorial variability and expression during degeneration; but it is highly questionable whether any further increase will occur from the future mating of the highest numbered chicks.

LEG. COVERTS.

In the newly hatched chick the outer surface of the fore-leg is provided with natal down feathers, similar to those over the wings and body generally (Pl. VI, fig. 3). They are merely tufts of barbs bearing barbules, without any shaft, and in due course are pushed out by the definitive feathers of the chick plumage. The pteryla usually persists until the chick is about six months old, but from this time onwards the feathers, complete in every way, gradually drop out without others taking their place. The first to disappear are those over the outer surface, followed by others along the sides, until when about a year old the leg is entirely naked with the possible exception of a few odd feathers along the posterior border, and rarely along the anterior border (Pl. VI, fig. 4). The rate of falling out and the number persisting vary in different birds. The pits of the empty sockets are visible for many years, gradually becoming less conspicuous¹.

¹ It may be noted that after the adult plumage is attained in the ostrich at two or three years, no external distinctions remain by which the age of a bird can be determined; the complete absence or otherwise of the contour feathers of the leg, as well as the freshness of the pits of the empty sockets, are some approximate guide for the first few years. Some farmers' birds are known to be between forty and fifty years old.

Were an adaptive significance for the loss of the leg coverts to be sought for it might possibly be found in connection with the increased size of the wing plumes during the middle chick stage. While the wing plumes are small the outer surface of the leg is exposed, and would be uncovered were it not for its own contours. As the chicks become older the wing-quills grow longer and, in extending backwards and downwards, serve to cover the fore-leg, when it may be held there is no further need for the protection from the contours.

Admitting an adaptive value of the above nature does not however imply any direct connection between the adaptation and the loss of the contours. Without doubt the relationship suggested is incidental, while the dropping out of the feathers is part and parcel of the general scheme of plumage retrogression going on in the ostrich. But the method by which the loss is achieved is of an altogether different nature from that followed in the case of the coverts and remiges. The germinal factors concerned with the production of leg contours would appear to retain their normal activity until the chicks are about six months old, and then by far the greater number lose their effectiveness and the feathers fall out, all within a brief period, due to a common influence acting simultaneously, much in the same manner as the human hair sometimes falls off after a severe attack of fever or of influenza. Two explanations seem possible. Either the feather-producing factors directly lose their effectiveness with the late chick stage, or they are inhibited by some physiological influence arising in the chick. Whichever be the case the original factorial change leading to the loss was presumably effected in the germ plasm, and is of the nature of a retrogressive mutational variation, although expressing itself late in ontogeny.

An ontogenetic loss of this nature has manifestly a wholly different significance from the successive disappearance of the individual feathers belonging to the rows of coverts and row of remiges, where each in turn retrogresses in a slow, successional fashion. The crural feathers drop out nearly simultaneously from a whole area, an entire pteryla is involved, and the losses are complete feathers which have not undergone any preparatory degenerative change, either as a whole or in their constituent parts. The one is as typical an illustration of continued, retrogressive change, with a definite trend, as could well be conceived, while the other is as direct an example of discontinuous, retrogressive change, unconnected with any other; yet both are to be found in the same organism, and represent the same tendency towards plumage reduction.

BALD HEAD PATCH.

The bald head is another character involving an ontogenetic loss of plumes of somewhat the same nature as that connected with the leg (Fig. 5). In this case however the loss affects only the North African ostrich and not the South African, and represents an accepted specific feature, whereas the disappearance of the leg contours occurs throughout the race. At the time of hatching the head of all ostrich chicks is covered, except over the pineal patch, with short bristly down, which later on is replaced by longer down with hair-like prolongations of the barbs. In the southern bird this covering persists, a still longer tuft

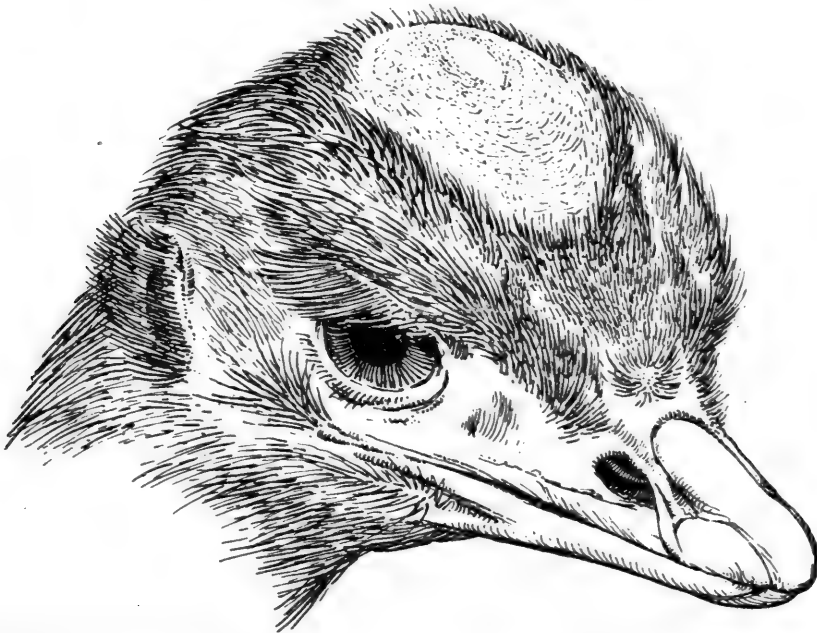


Fig. 5. Bald head patch of Northern ostrich. The clear oval area behind represents the pineal spot.

of bristly feathers often forming on the crown of the head. In the northern, on the other hand, the feathers begin to fall out when the chick is about three months old, and by six months an oval, bald patch is formed, varying somewhat in extent in different birds, and representing a definite mutation. The skin also becomes scurfy and the pits of the feather follicles disappear. In crosses with the southern bird

it has been established that the baldness behaves as a homozygous dominant, all the cross-bred chicks showing it at about the same age as do the northern chicks, and segregation takes place in the F_2 generation.

It may be presumed that in the past some germinal change took place in the northern bird as compared with the southern, as a result of which the feathers began to fall out from the crown of the head at a certain age. Whatever the change was, it is transmissible and behaves as a definite Mendelian character, dominant in crosses; the factor for baldness is dominant over one for a covering of feathers over a definite area. The factor however only becomes effective when the chick is about three months old (delayed dominance). It is a direct instance of factorial change occurring in a limited portion of the ostrich race, since no corresponding effect is shown by the southern bird. Naturally it is through such changes, restricted to certain parts of a race, that specific distinctions arise. All the other degenerative changes here described occur throughout the continent. If, following most systematists, we regard the southern and northern ostriches as distinct species then the degenerative changes occurring in both may be classed as "parallel mutations," presumably taking place in the parts of the germ plasm which are common to the two species¹.

The production of baldness in the ostrich, like that of the loss of the leg contours, has a different germinal and evolutionary significance from the other plumage losses hitherto noticed. Among the coverts, for example, the reduction takes place in a definite ordinal sequence, from one end or the other of a row, not a number at a time involving a special area; moreover, it takes place in piecemeal fashion and the factors are ultimately lost, the individual plumes no longer making their appearance at any stage of development. Presumably the changes

¹ Prof. T. H. Morgan (*l.c.* p. 47) has strongly emphasized that it is unsafe to judge as to the nature of the mutation from the appearance of the character alone; that the appearance of a similar character in related species does not necessarily represent a similar mutation, due to a change in the same gene or determinant, even though they may have many of these in common. He instances two different white-flowered races of sweet-peas which represent different mutations; three independent mutations have produced white birds; and by the refined method of localizing genes in the chromosomes establishes the different mutative origin of several of the closely related eye colours of *Drosophila*. All these however are concerned with such complex changeable characters as colour in which many factors are concerned, and it by no means follows that similar possibilities hold in the case of more fundamental structural parts of the organism. For these it would surely appear to be safe to assume that corresponding changes in related species take place in parts of the germ plasm which they inherit in common from the ancestral stock.

involved in the loss of the cephalic and crural feathers are germinal, though they only gain expression in the late chick. It may be surmised that both will gradually retrogress to an earlier stage in the ontogeny of the bird, the coverings first failing to appear on hatching and afterwards becoming wholly eliminated in the developing chick. A hastening of the time at which structures ontogenetically disappear is suggested in several other directions, particularly in connection with the digits.

The losses of feathers from the head and legs are obviously examples of the discontinuous variations of Bateson and of the mutations of de Vries, while all the other degenerative changes noticed are successional variations with a definite trend, illustrations of orthogenesis as understood by Eimer, and of the rectigradations of Osborn. The changes in the one are entire and detached, while in the other they are successional and cumulative, following a definite order. The former are examples of negative, germinal mutations, though expressing themselves late in ontogeny, and seem altogether fortuitous in their nature, whereas the latter appear to be parts of a definite, deep-seated, orthogenetic plan. The two types of variation are clear and distinct, and represent the two groups into which students of evolutionary genetics are often classed at the present time, namely, those who maintain that evolution takes place by large variations, mutations or saltations, and those who conceive it to be effected by minute, successional variations with a definite trend. Here in the same bird we have concrete examples of both types of variation, showing the danger of presuming that biological phenomena can ever be arranged in water-tight compartments.

DOWN.

In most flying birds a covering of down feathers or plumules occurs beneath the ordinary contour feathers of the body and wings, and a number of small, degenerate, hair-like feathers or filo-plumes may also be present. All accounts of the plumage of the ostrich however refer to the absence of down feathers and filo-plumes¹. Yet on close examination every Nigerian and Cape bird is found to display them around the base of the larger feathers on the wing and tail, the remiges and rectrices, the degree of development varying much in individual birds.

¹ Chandler (*Univ. Calif. Publ. Zool.* Vol. XIII. 1915, p. 284) gives a "total absence of differentiated plumules and filoplumes" as one of the characters of the Ratitae, as well as of the ostrich.

A casual examination of the surface of the body would fail to disclose them, but farmers often remark upon their presence on the wings and tail, and have some vague notion that a strong development is indicative of a good plumage bird. They are usually more sparse on northern than on southern birds, being almost absent from some individuals.

As regards the individual feathers some are fully developed though diminutive plumules, with quill, shaft, and flue, but by far the greater number are imperfect and degenerate. In place of a single shaft, there may be two or three imperfect ones; some are tufts of barbs provided with barbules, like the natal down, only smaller; while others are reduced to one or two hair-like barbs, with or without any barbules (Fig. 2). But all intermediate stages between the extremes can be observed, mingled together on the same bird. The remarks already made in connection with the degeneration of the coverts as regards the factorial changes involved apply with equal force to the down feathers, though here complete feathers are rare, and the degenerative processes largely concern the loss of the factors responsible for separate structural parts.

While usually restricted to the wings and tail, down is occasionally found to extend over a wider area. Some birds display a sparse covering of hair-like barbs over the whole of the hind part of the body, including the broad lateral apteria. The upper part of the wings may also be scantily provided, and in a few instances extremely degenerate down has been observed over the inner naked part of the wing, where it would least be expected, were it not for the evidence already presented that this was at one time fully provided with coverts.

All these occurrences strongly support the view that the ancestral ostrich was clothed with an under-covering of down feathers over the wings and body generally, in addition to the covering of large contour feathers, after the fashion of most flying birds. Usually it is now restricted to the area of the larger wing and tail feathers, but occasionally it spreads over the more distant parts of the body, including both pterylae and apteria. In addition, the individual feathers are now rarely typical down, complete with quill, shaft, barbs and barbules; they mostly exhibit the later stages of degeneration, ending in one or two hair-like barbs and then complete loss.

Two definite directions in the process of degeneration are thus indicated which take place concurrently, namely, a reduction of the area of the body provided with down, and a diminution and loss of the constituent parts of the individual feather. When completed the

latter naturally brings about the former, but the process has taken place more rapidly in some areas than in others, and so produced a regional difference. They correspond with the degenerative results among the coverts, where the constituent parts disappear in the same sequence and the area of the fore-wing provided with feathers gradually becomes less and less. On the view that the plumage of the ostrich as a whole is retrogressive, proceeding for the most part according to some definite order, the down might be expected to be the first to suffer, and the weak representation at the present time serves to confirm this. The many intermediate conditions still surviving indicate that the process has been a continuous one, while the extremely sparse remnants in some birds suggest that in time the covering will be entirely lost in certain strains and ultimately for the entire race. Embryos of all ages fail to afford any fuller ontogenetic traces of the down, so that when absent in the chick and adult it may be presumed that the factors concerned have altogether disappeared.

DIGITS OF THE WING.

The wing of the ostrich is usually described as degenerate compared with that of other birds. But the term can be applied only in a superficial sense, as referring to its size in contrast with that of the legs and body. The disproportion is such that in their present state the wings could never have been effective for flight, even if the loose plumes had enabled them to offer any resistance to the air. As a matter of fact, the detailed structure of the wing, particularly as regards the digits and their claws, proves it to be less degenerate than that of perhaps any other living bird, and to be more nearly related to the supposed reptilian ancestor of the class *Aves*. Evidence has already been presented to show that the fore-wing is probably still undergoing a gradual reduction in size, in correlation with the loss of plumage. It seems likely, however, that any change which occurs takes place in the wing as a whole, and is not necessarily accompanied by loss of its separate structural parts, as appears to be largely the case with *Apteryx*. In plumes also it is shown that a reduction in size as a whole takes place, and only towards the end a loss of the constituents. The two processes need to be kept distinct in retrogressive studies, in the same manner that we distinguish between progressive increase in size and in complexity. As regards the adult ostrich the third digit and its plumes are the only structures which seem to be in a degenerative phase at the present

time, and to afford details as to the manner in which the process takes place. In the embryo, however, many earlier stages are represented which indicate just as truly the manner of degeneration, even though the process is confined to the egg.

In all modern birds the third digit¹ is greatly reduced in size compared with the second and first, and is buried in the muscles of the wing. The first is also small, but is free and bears its own feathers, representing the ala spuria or bastard wing, while the second is axial and constitutes by far the greater part of the fore-wing. In the oldest known fossil bird, *Archaeopteryx*, all three digits were free and clawed, and perhaps bore feathers. In the adult ostrich the first and second are always free and clawed, while the third is unclawed and usually hidden; sometimes however it projects quite freely, and appears as a distinct third finger (Pl. V, fig. 2).

In the embryo the third digit is clearly seen at about the tenth day of incubation, and is then of equal size with the second; but from this stage onwards the latter greatly outstrips the former (Pl. VI, fig. 5), and within a few days of hatching the third shows only as a small, triangular projection from the post-axial border of the wing. After hatching it generally disappears and no trace is seen at the surface, but in some instances it persists, and may be altogether free from the rest of the wing—an approach to the ancestral free third digit (Pl. V, fig. 2). All intermediate stages between the extremes occur. Some writers assert that a claw appears on the third digit (Parker and Haswell, *Text-Book of Zoology*, 1910, Vol. XI. p. 421), but this has never been found in the hundreds of birds coming under the writer's observation, although looked for specially. As the ungual phalanx, which on the reptilian phalangeal sequence would be the fourth, has wholly disappeared the occurrence of the claw becomes most improbable. Judging from the retrogressive stages in the ostrich, the order of disappearance of the constituent parts of clawed digits is as follows: the claw first,

¹ In this and what follows the more usual method is adopted of regarding the bastard wing of birds as representing the first digit of the pentadactyle fore-limb and the other two as the second and third, following in this the views of Gegenbaur, Parker and others, as contrasted with those of Owen and Leighton, who consider the bird's digits as the second, third and fourth of the pentadactyle series. The conditions in the ostrich would appear to be conclusive of the former view, for the first digit bears two phalanges, and as the distal one is an ungual phalanx, no phalangeal reduction can have taken place. The second digit has the normal succession of three phalanges and the last bears the claw. Any loss of phalanges is from the distal to the proximal, and if a distal phalanx were lost the claw would disappear with it, as a more proximal phalanx would not become the ungual.

next the ungual phalanx, and then the other phalanges in regular succession from the distal to the proximal.

Dissections show that the first phalanx of the third digit is always well developed, but the second is usually represented only by a small vestige which is pointed at its distal end and fused with the first phalanx at the other (Fig. 6). In birds with the finger projecting

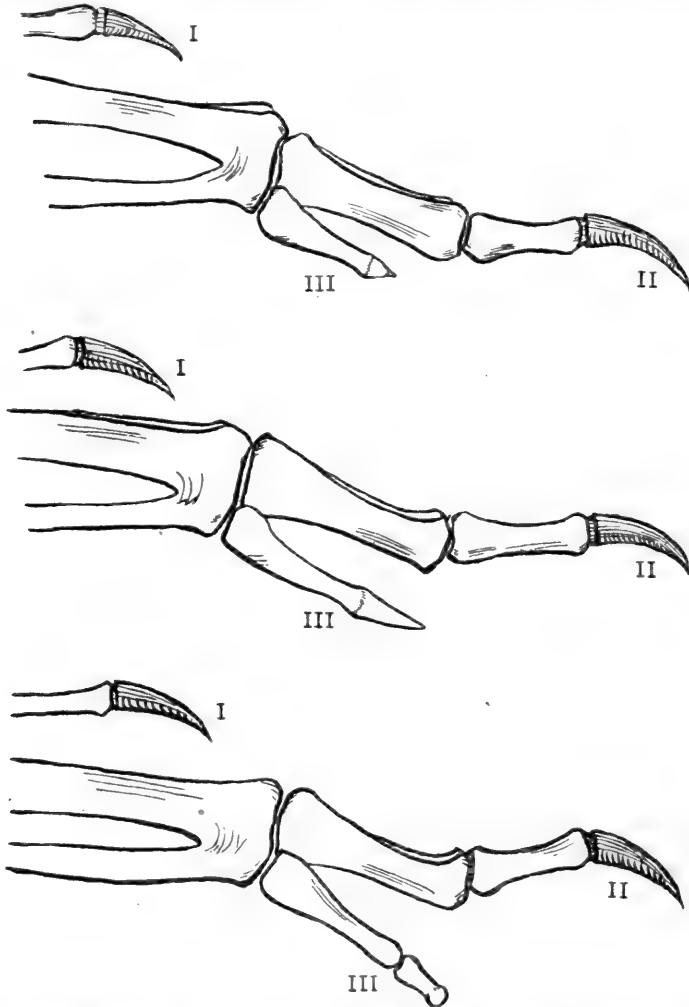


Fig. 6. Varying stages in the development of the second phalanx of the third digit. In the upper figure the phalanx is a mere knob, fused to the end of the first phalanx; in the middle figure it is longer, but still fused; while in the lower it is a distinct free bone, knobbed at its distal end, in correlation with the callosity on the skin (cf. Pl. V, fig. 2). I, II, III, first, second and third digits.

conspicuously the second phalanx is however a distinct bone, nearly an inch in length, articulating with the first, its free end knob-like in correlation with the callosity which it bears. But all intermediate sizes can be procured, corresponding with the varying sizes of the digit as seen from the outside.

Among the large number of ostriches available for study, we therefore find numerous variations which serve to illustrate how the degeneration of the third digit is proceeding, and these are supplemented by embryonic stages. In the adult no external trace of the digit may be left, while again it may project as a distinct finger; also the second phalanx may be represented by intervening stages from a mere nodule to a bone an inch or so in length. Different individuals have reached different stages in retrogression, but the passage from the one to the other is by barely perceptible stages, not by conspicuous steps.

In the embryo ostrich, as Dr R. Broom¹ has first shown, traces of the first three digits of the wing occur at about eleven days' incubation and also a vestige of the fourth metacarpal. The latter however persists for a very short time, and no trace of it appears in the late or newly hatched chick. The factors concerned in the partial production of the fourth manifestly still occur in the germ, but in a very reduced and weakened condition compared with the ancestral reptilian state, where the fourth and fifth were presumably fully formed and persistent. We may suppose that the genetic factors concerned with the third digit are equipotential with those for the second as far as the eleventh day of incubation, for both digits are then of the same size and each bears two phalanges. Later, the factors for the third digit undergo a comparative weakening and ultimately only one or at most two phalanges remain without any claw at the tip, whereas the second digit acquires its three phalanges and a claw. The factors for the third claw have wholly disappeared, while those for the second phalanx are almost lost in some individuals but fully retained in others.

With the series of embryos available, it would be possible to trace the loss day by day of the vestigial parts of the fourth and third digits as it occurs within the egg. It would merely serve to demonstrate however what is already fully established from the second phalanx of the third, that the reduction and ultimate loss proceed in a gradual, continuous manner. The digits have not disappeared at once in their

¹ "On the early development of the Appendicular Skeleton of the Ostrich, with remarks on the Origin of Birds," *Trans. S. A. Philos. Soc.* Vol. xvi. 1906.

entirety, nor have the various phalanges, but each has become slowly reduced in size. On the face of it the conclusion seems irresistible in favour of a gradual factorial weakening by changes of one kind or another. The factorial degradation may be so great that it gains somatic expression for only a brief period in the embryo and is not continued even to the time of hatching.

Plumes on Third Finger. The tip of the third digit, whether free or hidden, extends nearly to the hind border of the wing, and at this place one or two additional plumes sometimes occur, interfering with the regularity of arrangement of the row of under-coverts and wing-quills (Pl. V, fig. 2). These irregular feathers are often a puzzle to ostrich farmers, since from their size and position it is clear that they are neither remiges nor coverts. Dissections show that they belong to a distinct series, their internal quills crossing the phalanges of the third digit in the same manner as the quills of the remiges cross those of the second digit. Without doubt therefore they are to be regarded as belonging to the former, though being situated near its tip they at first give the impression of being under-coverts or wing-quills.

In addition to the feathers at the tip, single plumes are on rare occasions found set along the course of the buried digit. They may appear at any part of its length, and are of a distinct type from the under-coverts. It is submitted that the one or two at the tip, along with these single feathers, are survivals of a time when the ancestor of the ostrich had its third finger fully provided with feathers, in the same manner as the bastard wing. If this be the case, then the ostrich is apparently the only living bird in which the third digit still bears feathers, though with the present facts before us there can be little question that this was the condition in ancestral birds.

The persistence of such a far remote ancestral condition in the ostrich, altogether beyond what is found in other living birds, is somewhat remarkable considering the extent to which plumage degeneration in general has already proceeded. It is probably to be associated with the less degree of retrogression which the third finger has undergone. Along with the discovery of an extra-cranial pineal vesicle at a certain stage of development and a persistent pineal patch, it serves strongly to emphasize the primitive nature of the ostrich in certain respects; also the slowness of the retrogressive changes going on in some directions as compared with others, and their independence one of another. The independence becomes all the more impressive when we consider the degenerative stage reached by the digits of the wing compared with

those of the leg. We have in the ostrich a marked retention of archaic characteristics in certain directions along with a high degree of specialization in others. Structurally the digits of the wing are among the least degenerate of all living birds, while those of the foot are the most reduced.

FOURTH OUTER TOE AND CLAW.

The small, outer toe of the ostrich is the fourth of the pentadactyle foot, the first, second and fifth having already disappeared, though Dr Broom has shown there are traces of all five metatarsals in the embryo chick of ten and eleven days' incubation. At about two weeks' incubation the second toe shows as a very small projection, reminiscent of the three-toed ancestor (Pl. VI, fig. 5), and in the adult the distal end of its metatarsus remains as a small knob on the tarso-metatarsus, its proximal end being fully developed. Following upon the losses which have already taken place in the foot, the next toe in the sequence to disappear will be the fourth, and in the end the middle third will be the only one remaining, as in the case of the modern horse. Apart from the great difference in size between the fourth and third, other facts with regard to the claw and the scales on the upper surface give every reason for thinking that the former is in a retrogressive phase. Though small in size the normal sauropsidan number of phalanges—five—is still retained, the ungual phalanx naturally persisting so long as any vestige of the claw remains.

The scutellation on the upper surface of the fourth toe is short and continuous, only seven to ten scales being usually present, with little variation among the members of the race (Fig. 7). The scales cover only a small distal part of the digit, while comparison with other birds would lead us to expect they would extend the whole length, in continuity with those along the tarsus. It may be presumed that the factors for the missing scales have wholly disappeared from the germ plasm, for no evidence of them is ever forthcoming, even in the embryo. Along with the retrogressive claw, their absence is held to be so much evidence in support of the degeneration of the toe. As no differences of any moment however have been observed in the number, among either the northern or southern birds, it is probable they are not at present in a degenerative phase, in marked contrast with the claw. Evidence to be presented later, in connection with the scutellation over the third toe, proves that the scales do not drop out singly in an ordinal succession like the coverts and remiges, but in

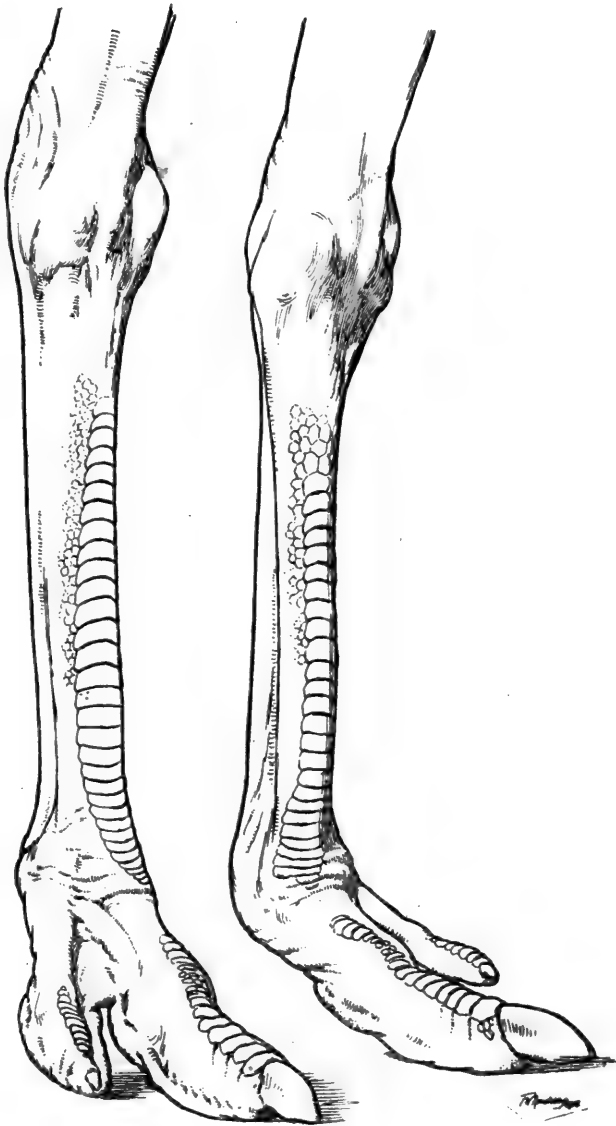


Fig. 7. Tarsus and foot of North African ostrich, showing arrangement of scales on the two toes; the small, outer toe also bears a claw. The scales have already disappeared from the first scutellar break and are broken up at the second.

groups at a time, giving rise to one or more breaks in the course of the row. In this case the seven to ten distal scales on the fourth toe do not represent the survivals of a larger series which has retrogressed one at a time from the original proximal end of the row, but a group which has not yet entered upon a degenerative phase; and it by no means follows they will retrogress singly, as would ordinarily be expected for meristic structures.

Already the claw on the fourth toe has for the most part disappeared from the race, though it is occasionally present, more often on North African than on South African ostriches. In 25 mixed northern birds it occurred on six specimens and was wanting on the others, while in 20 mixed southern it was found on only one. Everywhere it is feebly developed, especially by comparison with the big claw on the middle toe, and is always non-functional, never reaching the ground. Where best formed it projects for about half an inch from its socket, while at other times it is almost hidden in the integument, and can scarcely be felt with the finger; but all intermediate sizes between the extremes can be obtained. Usually it is equally developed on both the right and left foot of the same bird, though sometimes a difference is observed, or it may even be present on one side and absent from the other. In the present reduced state of the digit no adaptive significance can be attached to the presence or absence of the claw, and therefore natural selection is in no measure influencing its retrogression.

It may be observed that such a simple distinctive structure as a claw is easily seen and compared on different individuals, especially on young chicks which can be closely handled. The relative sizes readily admit of a grouping into strong, medium, weak and integumental, though without any sharp line of separation among them. As it is altogether functionless, never reaching the ground, any variation may be taken to represent a germinal as compared with an environmental change, and as due to intrinsic rather than extrinsic influences—a conclusion particularly convincing when a marked difference is apparent on the two sides. Moreover, the variations are just as conspicuous on newly-hatched or even unhatched chicks as they are on older birds. Since the claw is so frequently absent and varies so much in its development on different birds, it may be presumed to be in a highly degenerative phase at the present time. The results from the various breeding experiments are in like measure strongly suggestive of degradation being in progress, and will therefore be given in some detail.

Hitherto only one crossing has been secured from parents both of which are clawed and, as shown in Table IV, all four chicks obtained are also clawed. The clawed birds are usually found to be in a heterozygous or simplex condition with respect to the claw, in which case the mating of two clawed individuals should give progeny of which three-fourths would be clawed and one-fourth clawless. Naturally not much importance can be attached to a single experiment where the progeny are so few.

TABLE IV.

<i>Breeders :</i>	
North African cock, No. 252	Clawed
South African hen, No. 6	Clawed
<i>Chicks :</i>	
Four—Nos. 328 to 331 ...	All clawed

Where both parents are clawless the progeny also are usually unclawed, indicating that the genetic factors concerned are either wholly lost to the germ plasm or too weak to express themselves in the soma.

TABLE V.

<i>Breeders :</i>	
South African cock, No. 229	No claw
Cross hen, No. 233 ...	No claw
<i>Chicks :</i>	
Ten—Nos. 1 to 10 ...	No claw
<i>Breeders :</i>	
South African cock, No. 140	No claw
North African hen, No. 20 ...	No claw
<i>Chicks :</i>	
Six—Nos. 165 to 169, No. 172	No claw
<i>Breeders :</i>	
South African cock, No. 140	No claw
North African hen, No. 40 ...	No claw
<i>Chicks :</i>	
Eight—Nos. 231 to 238 ...	No claw

The results are not invariably so regular as in Table V when both parents are unclawed. A later hatching of eleven chicks from the first breeding pair above, Cock No. 229 and Hen No. 233, contained one chick with a tegumental claw on the right toe and none on the left, the remaining ten being all unclawed as in the earlier hatching; also, as shown below, out of ten chicks hatched from unclawed parents, nine were like the parents while one had a decided claw on each toe.

In these two cases we seem to be forced to the conclusion that though no evidence of the claw appears in either of the parents, the

Degeneration in the Ostrich

TABLE VI.

Breeders :

South African cock, No. 140	No claw
North African hen, No. 11	No claw

Chicks :

Nine—Nos. 242, 243, 302,	
303, 305, 310 to 313 ...	No claw
One—No. 304 ...	Clawed

genetic factors concerned are yet present in some form or other, and though normally dominant, are too weak to gain somatic expression; by some fortunate gametic redistribution or somatic condition they have been enabled to manifest themselves, in the one case on a single toe and in the other on both. Appearing in this fashion they represent "Reversions," as the term is employed by Bateson, C. B. Davenport and others; they are ancestral characters which have ceased to appear for a time, and then for some reason or other make their appearance. Apart from considerations of Mendelian "dominance" and "recessiveness," it is manifest that in cases where degeneration is going on genetic factors may be present in the germ plasm without necessarily expressing themselves in the soma; the character may or may not appear, dependent upon more or less incidental circumstances.

Where one parent is clawed and the other not, the claw generally appears in about half the progeny, showing that normally the clawed birds are dominant heterozygotes, which is what would be expected considering the small proportion of clawed to clawless individuals among the mixed assemblage of both northern and southern birds. Further, where the proportions in the progeny are approximately equal, factorial stability is suggested.

TABLE VII.

Breeders :

North African cock, No. 78	...	Weak claw
South African hen, No. 225	...	No claw

Chicks :

Five—Nos. 314, 316, 320, 322, 323	No claw
Four—Nos. 315, 318, 319, 321	Clawed

Here of nine chicks hatched, five were unclawed and four clawed. The same cock mated with a North African hen gave the like normal Mendelian proportion for heterozygotes, but in two cases with a marked difference in the size of the claw as between the right and left toes (Table VIII). A result of this nature and other similar ones given below seems to be clear proof that the genetic factors concerned with the claw are by no means of fixed or constant potency, but tend to

fluctuate, not only between individuals but also between the right and left sides.

TABLE VIII.

Breeders :

North African cock, No. 78	Weak claw
North African hen, No. 44	No claw

Chicks :

Three—Nos. 352, 353, 354	No claw
Two—Nos. 340, 356 ...	Right claw strong, left weak
One—No. 357 ...	Both claws strong

The varying manner in which the claws may express themselves in progeny from the same breeding pair is further illustrated in the next Table.

TABLE IX.

Breeders :

North African cock, No. 84	Claws medium
North African hen, No. 87	No claw

Chicks :

Two—Nos. 342, 343 ...	No claw
Two—Nos. 345, 346 ...	Claws scarcely showing, integumental
One—No. 241 ...	Right claw medium, left weak
One—No. 244 ...	Both claws medium

Where one parent is clawed and the other not, the Mendelian proportion for heterozygotes is not always maintained in the progeny, as shown below, where the hen of the previous series is mated with a different cock.

TABLE X.

Breeders :

North African cock, No. 284	Claws medium, but abnormally upturned
North African hen, No. 87	No claw

Chicks :

Six—Nos. 1 to 3 ; 5 to 7 ...	No claw
Two—Nos. 4, 8 ...	Claws medium and upturned
One—No. 9 ...	Upturned claw on right, none on left

Out of nine chicks hatched, only two were provided with full claws and one with but a single claw, instead of the Mendelian expectation of four or five fully clawed. The irregular, upturned form in the parent cock may possibly be an indication that the genetic factor was not of normal stability.

The variable result from an obviously weak factorial representation is still better illustrated in the following series. That the genetic factor was actually weak and on the verge of failing to express itself may be inferred from the fact that on the clawed parent the right claw was so

small as to be almost buried in the integument, while on the left toe it was wholly lacking.

TABLE XI.

Breeders :

South African cock, No. 226	No claw
Cross hen, No. 167	Claw on right toe integumental, no claw on left

Chicks :

Eight—Nos. 1, 3, 5, 7 to 11	No claw
One—No. 2	Integumental claw on right, no claw on left
Two—Nos. 4, 6	Integumental claws on both right and left

Out of eleven chicks eight have no claws, two have a very small one on both right and left toes, and one resembles the hen parent in having a small claw on the right and none on the left. Clearly the claw factor in the hen is in a weak fluctuating state, and either fails to gain its normal proportion of expressions in the progeny or has but a feeble result. We have a complete departure from the more or less rigid Mendelian proportions, but only what might be expected where the factors are in a variable, degenerative state.

Though the claw is normally dominant in heterozygous proportions, in the series below this is very far from being the case. Out of ten chicks hatched from the same clawed cock as in Table IX, but with a different hen, only one was provided with a claw, and then only on the right toe, not on the left. The progeny in Table IX represent a weak factorial expression, while below it practically fails altogether. As already established in connection with the wing-quills, Mendelian proportions are not necessarily maintained in crosses where degeneration is in progress. Factors or factorial systems near their limit of expression may or may not gain expression in the progeny, and so disturb the expected ratio.

TABLE XII.

Breeders :

North African cock, No. 84	Claw medium
North African hen, No. 116	No claw

Chicks :

Nine—Nos. 1 to 9	No claw
One—No. 10	Medium claw on right toe, none on left

From what is given above, there can be no question that the claw on the fourth toe of the ostrich is in a variable degenerative phase at the present time, while its rare occurrence in a mixed assemblage shows that complete loss has freely taken place in the past. The various degrees of expression in the chicks hatched are eloquent testimony of corresponding factorial variation in the germ plasm, so far as

we are able to interpret one in terms of the other. In some instances there is little or no departure from the Mendelian proportion for heterozygotes, and in these we may deem the genetic factors to be fairly fixed and stable for the time being, and at their present degenerative phase; while again we get a departure from the expected proportion and a diversity in the degree of expression which can be understood only on the assumption that the factors are in a variable phase.

In practically all cases the departures from the normal are retrogressive, indicating that the germinal changes are degenerative in their tendency. Where, as in Table VI, the reverse is the case it may be interpreted as revealing that the germ factors are still present, but in such a weakened state that expression is gained only under peculiarly favourable circumstances; for the many results in Table V show that where the claw is somatically lost expression is not likely to be regained. Both reversions and additional losses may be expected to occur as irregularities when the genetic factors are reduced to near the limit of expression.

Entirely independent of any extrinsic influence as the claw is found to be, the various sizes in which it expresses itself—strong, medium, weak, integumental—can only be regarded as proof that the factorial degradation is also of a slow, continuous nature; and it is reasonable to suppose that where expression altogether fails the genetic factors may yet be present in the germ plasm in various degenerative phases, though too weak to gain somatic manifestation. As in the case of plumage degeneration the appearance of the claw on one side and not on the other, or even a marked difference of expression, is also deemed to be evidence of fluctuating weakness on the part of the factors concerned.

SCUTELLATION OF MIDDLE TOE.

Along the front of the tarsus of the ostrich extends a series of large, nearly rectangular epidermal scales which in most cases pass uninterruptedly to the end of the big middle toe (Fig. 7). Over the leg the scales remain approximately of the same size, but at the junction with the toe they often become narrower, enlarging again distally. In flying birds generally it is found that the scutellation divides at the tarso-pedal joint, and is continuous with the smaller scales over the upper surface of the four toes. In the ostrich however, as noted in the previous section, a large interval invariably occurs between the tarsal scales and those on the small toe, the latter only numbering seven to ten and

situated distally. Taken in conjunction with the other facts of degeneration it is held that the rest of the scales on the fourth toe, which should connect the remaining few with the tarsal system, have been lost to the race, and are a phase of retrogression. It will be shown that the inception of a similar loss is taking place over the big middle toe, and may be also taken to represent its first expression of degeneration.

At the joint between the tarsus and big toe a definite break in the scutellation sometimes occurs, and very rarely a second break over the middle joint of the toe (Pl. VI, fig. 6). As in the fourth, the breaks represent a distinct loss of scales. They are clearly new retrogressive characters in course of introduction into the ostrich race. Out of 20 Cape birds of mixed breeding one only showed the discontinuity, while in 20 mixed Nigerian birds a single break occurred in three cases and a double break in two; thus its introduction has proceeded somewhat further in the northern than in the southern bird. Where two breaks occur only five or six scales remain between them, and should these disappear the big toe would have only a single distal series, about seven to ten in number, and its scutellation would then be altogether similar to that characteristic of the small one at the present time. Therefore the stages represented by the big toe may in all likelihood be taken to illustrate the manner in which the reduction in scales was effected on a small toe, though the latter has retained no confirmatory survivals. The losses have probably no adaptive significance whatever and therefore no selection value. They are found on chicks on hatching and while in the egg, and remain unchanged during the ontogeny of the bird.

Where numbers of ostriches are taken into account no sharp line of demarcation is to be found between the continuous and the discontinuous scutellation on the big toe. In some scarcely any narrowing of the scales occurs at the first joint and none at the second, and from this all stages in their reduction can be observed, first over one, and then over both joints, their place being taken by the insignificant scales which cover the lower part of the leg generally. As in former degenerative series the many variations encountered are deemed to represent the order according to which the retrogressive changes take place for the race as a whole. An unexpected difference however obtains in the manner of disappearance of the scales over the first joint as compared with those over the second. In the former they simply become smaller and smaller until they cease to be continuous, and then ultimately disappear, the middle smallest going first, and the others in a regular

ordinal succession in both directions. In the latter each large scale first fragments into a number of irregular, smaller ones, and these then gradually diminish until they vanish altogether, and a clear interval separates the middle and the distal series (Fig. 8). Thus we have the

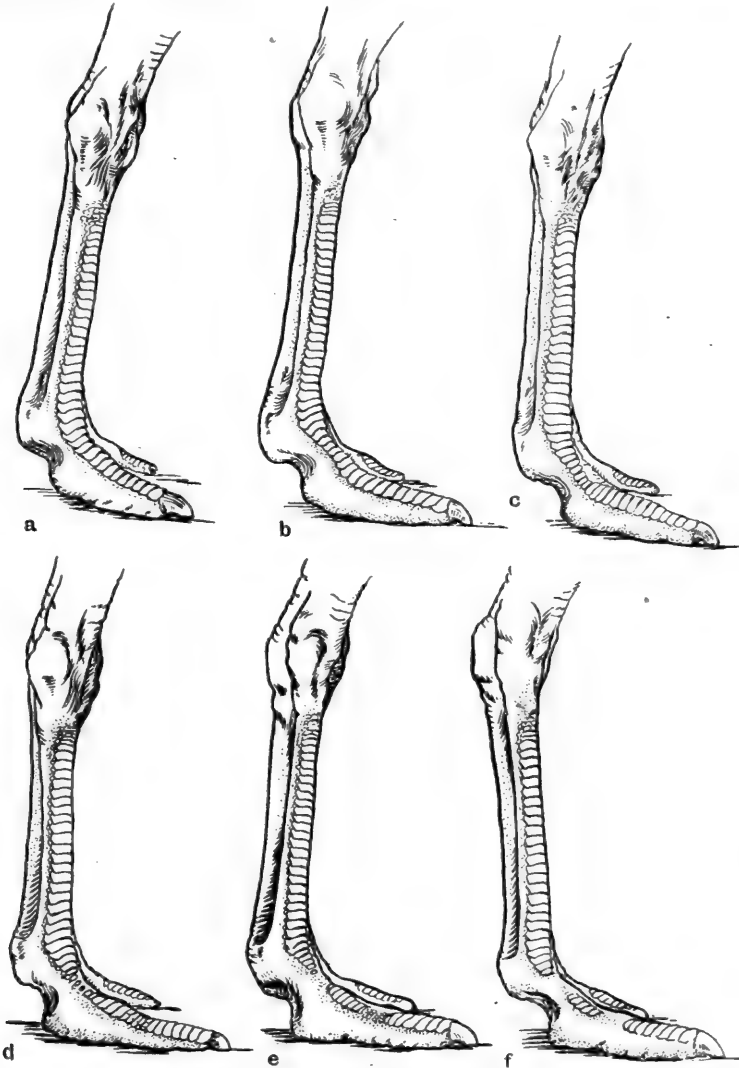


Fig. 8. Series showing the various stages in reduction of the scales on the large third toe. In the three upper a gradual narrowing is seen over the first joint; but no change over the second; in the three lower the scales over the first joint gradually disappear, while those over the second joint undergo fragmentation and then disappear.

phenomenon of two wholly similar characters arising by quite different methods. In the following description an "incomplete" break is understood to be one in which the scales over the joint are so reduced as to separate or become detached from one another, but where vestiges still remain. A "complete" or "entire" break is one in which a clear interval occurs over the joint, free of scales, except of the kind over the leg generally.

A break is manifestly a single, complete act of degeneration, representing an entire loss of about seven or eight scales over the first joint and four or five over the second, and the part reduction of the others in each direction towards it. All stages in the process are forthcoming, which show it to be gradual and sequential, the method being different for the one break as compared with the other. An almost simultaneous loss of a group of structural elements from a linear meristic series, at some part other than the ends, offers a great contrast to the manner in which the reduction of the coverts and remiges is effected. In the latter, successive individual losses occur from one or both ends of a linear series, in place of a disappearance from an intermediate part. Differences of this kind show that any assertion as to the order or manner in which past losses have taken place, without supporting evidence from the intermediate steps, as well as any prognostication as to the course which will be followed in future losses, is a proceeding attended with many uncertainties.

The breeding experiments serve to establish that a break is germinal in its origin, and represents a dominant Mendelian character, an absence dominant over a presence. Where no interval occurs in either of the parents the progeny in nearly all cases reveal a like continuous scutellation, showing that the influence responsible for the break is wholly absent or non-effective. Thus in twelve different hatchings, where neither the cock nor the hen showed any break, each of the 71 chicks obtained had the tarsal and digital scales continuous. In these cases the factors concerned in the production of the scales are manifestly in a fairly fixed and stable homozygous condition in the parents, and we have uniformity of somatic expression in the progeny. The stability is however by no means so constant as might be expected, for in most of the series much variation is to be found in the degree of narrowing of the scales over the first joint, even on progeny from the same parents.

Occasionally however an incomplete or complete break may occur in the progeny of parents, both of which show no signs of any, as in the two series given below. (Tables XIII and XIV.)

TABLE XIII.

<i>Breeders :</i>	
South African cock, No. 140	No break
North African hen, No. 20	No break
<i>Chicks :</i>	
Three—Nos. 165, 169, 172...	No break
One—No. 168	No break, but scales very narrow
One—No. 167	Break, incomplete
<i>Breeders :</i>	
North African cock, No. 78	No break
North African hen, No. 40	No break
<i>Chicks :</i>	
Five—Nos. 352, 353, 354, 340, 357	No break
One—No. 356	Break well-defined

Since the evidence to be presented serves to establish that the scales over the third toe are mostly in an incipient degenerative phase, the inherent tendency may well be expected to become intensified at times and to manifest itself in the chicks, though not somatically apparent in either of the parents; and it is probably as racial losses sustained *de novo* that cases like the above, and others presented below, find their interpretation. In general the factors concerned in the appearance of the scales at the joint are so stable that no actual break in the progeny appears, though there may be much variation in the degree of narrowing; but in some birds they may be in a further degenerative phase, near the "breaking point," as it were, and a combination of intrinsic circumstances may give an incomplete or complete break in one or more chicks, though not indicated in the parents.

Where one of the parents displays the break and not the other, then, as shown below, the factor for the break acts as a simplex dominant, and the interruption occurs in half the progeny. The heterozygous condition is what would be expected considering the small proportion of

TABLE XIV.

<i>Breeders :</i>	
North African cock, No. 78	No break
South African hen, No. 225	Break
<i>Chicks :</i>	
Five—Nos. 314, 315, 321, 323, 301 ...	No break
Six—Nos. 316, 318, 319, 320, 322, 302	Break
<i>Breeders :</i>	
North African cock, No. 252	Break
South African hen, No. 6	No break
<i>Chicks :</i>	
Two—Nos. 328, 330	Break
Two—Nos. 229, 331	No break

birds which possess the break. Even if the break factor first appears germinally as a duplex dominant¹ the mating would in all probability be with a nulliplex which would give simplex dominants. Until therefore the break appears in a much larger proportion of the race the individuals showing it in a mixed assemblage are likely to be simplex.

The different stages of formation of the break may be as distinctively germinal and transmissible as the completed break itself, and approximately follow the proportions for heterozygotes. This is clearly established in the series below (Table XV). The parent hen, No. 167, bred as shown in Table XIII from parents both without any break, displays an incomplete single break, only a few vestigial scales serving to connect the tarsal and digital series. Among the eleven progeny obtained, five display scutellar continuity like the parent cock, five have an imperfect break like the parent hen, but varying somewhat in degree, while one has a clearly defined break, representing a further stage of retrogression than any of the other chicks or either of the parents.

TABLE XV.

Breeders :

South African cock, No. 226	No break
Cross-bred hen, No. 167 ...	Incomplete break

Chicks :

Five—Nos. 1, 2, 5, 8, 11 ...	No break
Five—Nos. 4, 6, 7, 9, 10 ...	Incomplete break, variable
One—No. 3	Complete, well-defined break

In this series there can be no question that we are witnessing the loss of scales in actual progress at the tarso-digital joint. While the factors involved in their presence or absence are for the most part stable, we also find fluctuations possibly due to the incoming of new "break" factors, and even a complete loss may appear without any indication of such in either parent.

The fluctuating nature of the factors is still better exemplified in the next series (Table XVI), as also a marked accentuation. In the cock only a slight narrowing is displayed over the first joint, but in the hen an

¹ As regards the homozygous or heterozygous condition in which new characters first appear Prof. de Vries remarks as follows in a short article (*Science*, Vol. XLVII. May 10, 1918) on "Mass Mutation in *Zea Mays*": "It is now generally conceded that mutations take place ordinarily in the production of the sexual cells, some time before fecundation, probably at the time of synapsis. From this conception the conclusion directly follows that the copulation of two similarly mutated gametes must be rather rare. Far more frequent must be the instances in which a mutated sexual cell combines with a normal one."

incomplete break occurs over both joints, the scales being vestigial over the first and fragmentary over the second.

TABLE XVI.

Breeders:

North African cock, No. 84	No break, slight narrowing at the first joint
North African hen, No. 116	Two incomplete breaks; first vestigial scales, second fragmentary

Chicks:

Three—Nos. 1, 3, 10 ...	Two breaks, both well-defined
One—No. 8	One break well-defined, scales over second joint, scarcely narrow
Three—Nos. 2, 4, 7 ...	No break, but scales very narrow at both joints
Three—Nos. 5, 6, 9... ..	No break; first joint scales very narrow, second scarcely narrow

Four successive stages are here represented in the breaking-up of the digital scutellation. Three chicks display two very decided breaks without any intervening small scales; one shows a well-defined single break only; three have no break, but the scales over the first joint are very narrow and are beginning to fragment over the second; while three have the scales at the first joint very narrow, but scarcely any change over the second. In each chick there is an accentuation, on definitely prescribed lines, of the breaking-up compared with that in the parent cock, while the three with two breaks represent an advance on the stage reached in the parent hen. If we admit that the incoming of some inhibitory or break influence is in progress, the accentuations can be readily understood, as well as the departures from the expected Mendelian proportions.

An accentuation in the loss of scales is still more marked in a case of in-breeding. The series is as follows:

TABLE XVII.

Grand-Parents:

North African cock, No. 9	No break
South African hen, No. 225	Break

F₁ Chicks:

Three—Nos. 180, 181, 182	No break, but narrowing
One—No. 179	Break complete on one toe, incomplete on other

Parents:

Cross-bred cock, No. 182 ...	No break, but narrowing
Cross-bred hen, No. 179 ...	Break complete on one toe, incomplete on other

F₂ Chicks:

One—No. 4... ..	No break, but narrowing
One—No. 2... ..	Single break
Two—Nos. 1, 3	Two breaks, well-defined

A series of chicks later than the above has been obtained, but they are not so reliable for purely genetic purposes seeing that the cross-bred

cock No. 182, was mated with hen No. 110, as well as with hen No. 179, and both laid eggs and assisted in the nesting, sitting side by side. The chicks are therefore from two sisters mated with the same brother, but the scutellation is slightly different in the two hens. The series is presented as showing practically all the intermediate phases in the production of the two breaks, mostly with marked accentuation on the parental conditions.

TABLE XVIII.

*F*₁ Breeders :

Cross cock, No. 182	No break ; narrowing at first joint, none at second
Cross hen, No. 180	No break ; narrowing at first joint, none at second
Cross hen, No. 179	Break complete on one toe, incomplete on other

*F*₂ Chicks :

One—No. 6	...	No break ; slight narrowing at first joint, none at second
Two—Nos. 2, 4	...	No break ; medium narrowing at first joint, none at second
Two—Nos. 5, 8	...	No break ; very narrow at first joint, fragmentation beginning at second
One—No. 1	...	Complete first break ; no fragmentation at second
Two—Nos. 3, 7	...	Complete first break ; scales fragmentary at second
Two—Nos. 10, 11	...	Complete first break ; scales strongly fragmented at second
One—No. 9	...	Complete first break ; complete second break

As arranged above the eleven *F*₂ chicks present an almost complete series of scutellar changes, comprising all the intermediate stages from a slight narrowing at the first joint to one where the first and second breaks are fully completed (cf. Fig. 8). The scales over the first joint simply become smaller and smaller and ultimately disappear ; before this occurs those over the second joint begin to break up and the fragments become less and less until no trace of them remains. Like those in Table XVII they demonstrate that the individual losses may represent a considerable advance on the maximum displayed either by the parents or the grandparents. It is difficult to resist the conviction that we are here witnessing the loss of scales in actual progress, and that by means of selection we are able to accentuate retrogressive evolution.

The two breaks constitute a most decided example of a similar somatic character being produced by two wholly different factorial methods. They justify the remark of Morgan that "experience has shown that it is very unsafe to judge as to the nature of the mutation from the appearance of the character alone," quite as conclusively as any of the instances he himself adduces.

Comparison may be made with the retrogressive changes in the plumes. In these a long process of simple reduction in size is followed by a breaking-up and ultimate loss of the constituent structural parts, while in the case of the scales over the second joint the first evidence

of retrogression is the breaking-up followed by the diminution and eventual loss of the fragments. Throughout we appear to have these two processes, namely, slow successional reduction in size and somewhat rapid fragmentation.

Though representing a somatic absence, the factor for the break is dominant over the factor for the presence of the scales, in the same manner as the factor for the bald head patch in the northern ostrich is dominant over that for the presence of the feathers in the southern bird. Discussing the "presence and absence hypothesis" Morgan¹ shows that in certain cases a "present" factor may be recessive. "In the cross of horned by hornless sheep, the horned condition dominates in one sex and the hornless in the other. Here no matter which is considered as a presence it must be conceded that in one sex or the other it is recessive. The view that dominance of a factor proves its presence and recessiveness its absence should therefore be abandoned."

DEGENERATION AND ADAPTATION.

A brief enquiry may now be made into the adaptational significance of the various degenerative changes which have been effected already in the ostrich and of those which are still in progress. First, as regards the losses of plumage. It is very questionable if those already carried out, such as the loss of down, leg feathers and many of the wing coverts and remiges, have any appreciable bearing on the welfare of the bird. In life under arid conditions any reduction in the covering will assist in radiation from the body, and may possibly be of some advantage in the intense heat of the day. During the hottest weather chicks and adults are often seen standing with their wings outspread and all the wing and body feathers erect. On the other hand, loss of plumage will reduce the protection against cold nights, though the overlapping of the present feathers still suffices to clothe the bird when crouching.

The ostrich is highly responsive to humid cold as contrasted with dry, cold weather. Its physiological state becomes greatly reduced during prolonged, cold, rainy conditions, and chicks especially often succumb to exposure, and even adults; or, if the exposure be during the breeding season, the cock may cease to pair and the hen to lay eggs, or both may stop nesting. But this is scarcely a consequence of the scantiness of the plumage, as might at first sight appear, but rather of the fact that

¹ Morgan, T. H., et al., *The Mechanism of Mendelian Heredity*, Holt and Co., New York, 1915, p. 221.

having no oil or preen gland the loose feathers absorb and retain water instead of allowing it to run off as in the familiar duck. An ostrich exposed to rain presents a bedraggled and sorry spectacle, its plumage clinging to its body like a veritable "wet blanket." The effects of exposure are mainly felt at low levels, subject to continued, cold coastal rains: at high elevations rains are infrequent, and are mostly short, sharp, thunder showers with rapid return of sunshine. A more complete covering would therefore be no remedy against the former conditions, but rather the reverse. The oiling of the feathers from a preen gland would alone suffice, and also prevent the high depreciation in the feather crop consequent upon its becoming wet and muddy, as well as that of the graceful millinery plume when exposed to humid weather.

At first no close connection would appear to exist between two such widely distinct phenomena as the geographical distribution of the ostrich and the absence of a simple structure like an oil gland. Yet from the above considerations it is manifest that the inability to oil its loose feathers limits the bird to an arid climate, and also restricts successful ostrich farming to the same conditions, as many a farmer has found to his sorrow in attempting to introduce the bird to humid areas. Under the circumstances, it will readily be admitted that the extensive plumage losses which have already occurred have little or no adaptive significance, and therefore little or no selection value; climatic conditions vastly outweigh in importance any loss of plumage hitherto, and particularly the slight variations between one individual and another.

As regards the small size of the wings, no longer of use for flight, the welfare of the bird would suffer to a certain degree by their continued reduction, though probably no disadvantage has occurred hitherto. They are of much assistance in steering the bird in its erratic movements, particularly when twisting and turning, and also in its courageous but none too graceful waltzing display. When raised and lowered, either together or alternately, the wings enable the bird to perform rapid turning movements with remarkable agility, actions which may be deemed to be of some significance in escaping from the measured spring of the larger carnivora. The wings are also necessary in the frequent practice of taking a "sand bath," when they throw the dry sand and dust over the body of the bird, a doubtful remedy against skin and feather parasites. During natural incubation they are essential for covering the large nest of eggs. On the whole, however, we may hold that the wing reduction hitherto has had no harmful result, but should it continue a stage will be reached when it will have a negative selection value.

It may be held that some mechanical advantage to the bird has accrued from the loss of the three toes in the matter of rapid movement, and that this will be increased still further when the small fourth disappears: for then the bird will have the same single-toed foot as the horse, which many writers deem to be a forcible example of a purposeful adaptive change. But if the interpretation here given of the loss of scales from the third toe be correct, namely, that it is the first hint of a degeneration which the toe is about to undergo, then retrogression will not cease with the disappearance of the fourth, and the temporary advantage from the possession of only the middle toe will ultimately be changed to one of real disaster by the continued action of the same degenerative force; certainly a vital argument against the notion of any purposeful significance in the retrogressive changes.

On the whole we are forced to conclude that no necessary connection exists between the degenerative losses in the ostrich and the welfare of the bird; they are changes which have taken place apart from any adaptive considerations, and in the main are indifferent as regards the every-day life of the bird. Incidentally they may come to have an adaptive significance, but it is just as likely to be harmful as beneficial.

During the course of its existence, even from the egg stage onwards, the ostrich is so surrounded with dangers that, compared with them, any slight structural variation must have an infinitesimal selection value. The position of the nest, often containing scores of eggs, is never determined by any concealing or protective consideration; jackals and hyaenas show a wonderful aptitude in rolling the eggs about until they break, or of being on hand as the chicks hatch, hardly deterred by the frantic display of the parents to drive them away. Some slight parental care is manifested for the chicks as a whole, but an utter indifference to the fate of the individual. A spell of cold, wet weather in the absence of an oiled compact plumage is likely to be fatal; parasites, such as tape-worm and strongyles, abound and play havoc during any reduced state of nutrition, as from a period of drought; aspergilliosis has prevented many a farmer from rearing a single chick season after season; and in nature carnivora, large and small, are always at hand to exact their toll. With all these chances against the attainment and continuance of adult life it seems absurd to maintain that a slight reduction in the number of plumes, a lessening of the number of scales or the claw on the toes, or a slight diminution in the size of a phalanx could count as having survival values. Further, on the wide expansive plains of

Africa, a mutual struggle for existence is inconceivable; "either a feast or a famine," applies to the ostrich as to many other things in Africa.

If the losses have little or no bearing on the welfare of the ostrich natural selection has necessarily been inoperative in directing their course; they are wholly intrinsic and independent of any external modifying cause. Moreover, as the same mutative changes are going on concurrently throughout the race, no important individual differences will arise such as would afford material on which natural selection could be exercised; natural selection may wipe out the race, but can not guide its evolution. The slight differences in the rate of retrogression which afford us the details of the manner in which it is proceeding are altogether too insignificant to count in the broad, competitive life of the ostrich. Natural selection is powerless to check permanently the relentless, degenerative influences which are at work, and when the retrogressive changes have gone sufficiently far to interfere with the activities essential to its existence the ostrich will disappear as a race.

ONTOGENETIC AND PHYLOGENETIC DEGENERATION.

Factorial changes are held to be effected during the complicated mitotic processes of gametogenesis and fertilization which intervene between one generation and the next, and the hereditary differences in the new generation are determined at that time, and are uninfluenced during ontogeny. The conceptions underlying the phrase "non-inheritance of acquired characters" apply equally well to structures in a changing state as to those which are fixed and stable. The process of degeneration is in no ways effected during the life-time of the individual, but only with the formation of the zygote. On embryos and newly hatched chicks the plumes, scales, and claws are to be found in exactly the same degenerative phases as in the adult.

From the many directions along which degeneration has taken place several considerations emerge when the changes are viewed in their relationships to ontogeny and phylogeny, such as the question of the ontogenetic period of factorial expression, and also factorial persistency and atrophy. Whatever be their nature the Mendelian factors are held to reside in the germ cell, and to be part and parcel of it; we can also suppose that they exercise their influence upon the developing organism as part and parcel of the somatic cells¹, and ordinarily maintain it

¹ Prof. T. H. Morgan when discussing Weismann's *Praeformation Hypothesis* and the Factorial Theory in his *Mechanism of Mendelian Heredity* (p. 225) remarks as follows:

throughout life. But where structural parts hereditarily disappear during ontogeny we appear to be justified in supposing that some factorial weakening or loss is indicated, leaving aside any modifying inhibitory influence.

The loss of plumage from the head and legs takes place only several months after hatching. At the beginning the germinal factors concerned are present in all the members of the race, but persist in their full expression for about three months and six months respectively, afterwards undergoing some change which in the end leaves the areas featherless. We have a factorial germinal change taking effect within the life-time of the chick, but whether from atrophy, or inhibition, or a factor for baldness is not determined; the last is however suggested seeing that in the case of the bald patch the loss is dominant in crosses. A normal, late ontogenetic expression of factors is not unknown in the ostrich as in other animals, for we may recall the changes in the plumage, body colours, and general activities which coincide with the advent of sexual maturity. Though held to be determined by secretions from the gonads the true distinctive nature of these is dependent upon genetic factors late in expressing themselves, in the same manner as for the cephalic and crural feathers.

As regards the loss of plumage from the wings the germinal factors concerned become wholly lost to the individual in the end, no evidence of the plumes appearing in the embryo. They have undergone their slow, phylogenetic degradation in the mitotic passage from one generation to the other, and are absent from the beginning of ontogeny, that is, from the zygote. The claws and scales appear at a rather late stage within the egg, and persist in the same relative degree of development throughout ontogeny, but the factors undergo reduction in the passage from generation to generation. Apart from Mendelian recessiveness, failure of somatic expression for feathers, scales, and nails, is however no certain proof of the germinal absence of the factors. As they undergo gradual degradation a time will necessarily come when they reach the margin of somatic expressibility, but when this is passed the factors will presumably still continue their degenerative process, generation after

“Weismann still adheres nevertheless to his mosaic theory of development, but as before stated the modern work on development does not support this interpretation of development. His view assumes disintegration of the germ plasm when the body cells are produced in order to account for the localization of characters; the other view, following the experimental results and microscopical observations, assumes, so far as the chromosomal materials are concerned, that all of the hereditary factors are present in every cell in the body.”

generation, until final atrophy is reached. Thus the germ plasm of the ostrich may contain numbers of factors at various stages of degradation without any manifestation of them in the soma. The germ of an organism in a degenerative phase may be a very different matter from the evidence we have of its nature in the soma.

The factors concerned with the lost digits of the wings and legs are not yet entirely lost to the germ plasm of the race or of the individual, but only fail to continue their manifestation to a late ontogenetic period. As already shown traces of at least four digits occur in the wing after about ten days' incubation. The metacarpal of the fourth has but a brief persistence; the second and third are for a time co-equal in their expression, but the latter never completes its development and varies much with regard to the second phalanx. Traces of the five digits or their metatarsals occur in the foot of the ten days' chick. The first and fifth have a very transient ontogenetic existence, the second remains much longer, while only the third and fourth continue as digits throughout the life of the bird.

Although we assert that the ostrich has only three digits to the wing and two to the foot the remark should be accorded a time qualification. At a certain stage in its ontogeny the bird has hints of four and five digits respectively. Expressed factorially we should say that the genetic factors for the digits are present in the zygote; some gain somatic expression for only a brief, transient ontogenetic period and others persist for the life-time of the bird. For the former we may hold that some ontogenetic weakening or inhibition takes place and they cease to be effective beyond a certain stage, but the factors have by no means wholly disappeared from the germ, as in the losses of the remiges and coverts.

The persistence of factors only in the embryo stage, but presumed to have been effective throughout the ancestral phylogeny and in a form resembling other living types, is manifestly the Mendelian manner of expression for the principles represented by the Biogenetic Law of von Baer and the Theory of Recapitulation of Haeckel—the individual in the course of its development passes through stages corresponding with its course of evolution; ontogeny repeats phylogeny. This means that the factor or factors for a structure now degenerate still persist in the germ plasm, but in a degraded form, so reduced that they find expression for only a short period in the early stages of ontogeny; or perhaps the factors which represented the later ancestral feature have completely degenerated, and only the earlier ones persist. There is not a throwing

back towards the egg of the ancestral phylogenetic stages, as seems sometimes to be understood, but an inhibition or failure to continue somatic expression beyond a certain stage. What that later expression was can only be surmised by comparison with what are now regarded as related types; but we have no certain knowledge that our interpretation is correct; it may have been something beyond our experience of to-day. Ontogeny does not repeat phylogeny; any early structural similarity among phyla is only evidence of community of germ plasm as far as the two continue to express themselves alike, but not beyond. Ontogeny can only repeat phylogeny as far back as no factorial losses have occurred. In the case of a retrogressive character it does this to a less and less degree with succeeding generations. Once factorial losses have taken place throughout the race we have no certain means of knowing what this expression was; we can only surmise from closely related types. The germ plasm of all chordates carries the genetic factors for visceral arches and gill-clefts; they persist in their expression throughout life in the case of fishes and some amphibians, but lose it at an early developmental stage in sauropsida and mammals. We presume that ancestrally they continued longer, but have no certain proof thereof. The relationship involved is almost certainly that of community of factors so far as they have a common expression, but no further. Degeneration may be defined as the somatic expression of a phylogenetic degradation and loss of genetic factors.

The degenerative results in the ostrich further reveal that the factors for different classes of somatic structures tend to have different rates of persistence in the germ plasm. Superficial skin structures, like feathers, scales and claws, are rather late in gaining ontogenetic expression compared with more fundamental structures, like limbs, the skeleton, nervous system, and sense-organs. Likewise in degeneration the factors for the former readily disappear from the germ plasm, while those for the more fundamental parts, as exemplified by the digits, persist much longer, and disappear with extreme slowness. As a general relationship we may say that superficial or skin structures are late in phylogeny as also in ontogeny, while fundamental structures are early in phylogeny and in ontogeny; in degeneration the genetic factors for structures late in phylogeny and ontogeny early disappear, those for structures early in phylogeny and ontogeny are late in disappearing.

CAUSE OF DEGENERATION.

No attempt has been made to arrive at the cause of the degenerative processes which have been in progress for such long ages in the ostrich, and seem likely to continue for ages yet to come. The term "degenerative influence" has been mainly employed where necessity arose for appealing to some agency, as being altogether indifferent in its significance. The fact is we are altogether ignorant of the means by which, under ordinary conditions, changes can be effected in the germ plasm and become manifest in the soma, but a few considerations cognate to the matter may be noted.

In the first place, whatever the cause may be, there need be no question that it is an altogether internal or intrinsic one as contrasted with one environmental or extrinsic. The same changes are going on throughout the continent, with all its varied geographical and climatic conditions; the sequences followed and the stages reached are approximately the same everywhere; there is an entire absence of anything aberrant or fortuitous. All these point to something common to the race, and altogether beyond environmental agencies. The influence is so slowly acting, and has continued for so many ages and ages, as to call for an aloofness, an independence of external vicissitudes. Only something inherent within the organism itself and beyond all varying somatic responses could meet demands so continuous and consistent. The germ plasm alone satisfies the call for racial continuity. With our present-day conception of its continuity, the germ plasm supplies us with a vehicle which admits of a uniform influence being carried on uninterruptedly for countless generations. In fact degeneration in the ostrich seems to be as much a part of the race as does the germ plasm itself, and the one may well be part and parcel of the other. No evidence whatever is afforded that the changes are instituted *because* of any direct bearing on the welfare of the bird, or to meet any outward set of conditions; any adaptive relationship arising is wholly incidental. The direction of the changes comes entirely from within, there being the greatest improbability of any guiding trend from natural selection.

One of the most impressive features of the degenerative changes of the ostrich is the determinate nature of the influence at work, the absence of anything fortuitous, as if it were carried on generation after generation according to some definite, pre-determined plan. The ordinal succession of losses of the coverts and remiges, the gradual loss of the down, the regular sequence of losses of the digits of the wing and foot

are all convincing testimony of some consistent force, to all appearances acting apart from any environmental influence. They are beyond the ordinary germinal variations which a mixed assemblage of individuals of a species presents. It was evidence of this nature, but in a progressive direction, that led Nägeli to think of a mystical, internal, vitalistic force as directing organisms, and others to postulate an "inner directive force," an "inner law of development," or an "intrinsic tendency towards progress"; and in the degenerative phenomena of the ostrich we seem confronted with facts similar to those for which these phases stand, to-day regarded with so much suspicion. Morgan (*l.c.*, p. 50) remarks: "an orthogenetic series of changes does not in itself, without a closer analysis than has as yet been furnished, establish that an *innate principle, urge, vis-a-tergo, or driving 'force'* is causing the successive moves. The genetic evidence from multiple factors must create at least a strong suspicion against the 'will to believe' in the mystic sentiments for which these terms always stand. That a progressive series of advances in a gene might take place with a consequent advance in the many characters involved is *thinkable*, especially if it could be shown that environmental changes cause parallel progress in the gene and this in the character."

It may be suggested that instead of being due to some mystical, vitalistic, directing force the changes in the ostrich are to be interpreted in terms of germinal senescence, perhaps expressing itself in factorial fractionation and loss. It is averse from all our experience of nature to think of anything as fixed and immutable, and much more is this the case when considering organic matter, the most unstable of all. We are schooled in the senescence of protozoan and somatic cells, but the long contemplation of the phrase, "immortality of the germ plasm," has tended to divorce our ideas of the germ-plasm and somato-plasm, as though they were two distinct kinds of proto-plasm wholly apart. Just as there have been periods of "momentum in evolution," when certain groups progressed even to bizarre degrees, presumably as a result of germinal virility and exaggerated factorial change, so there have been periods when groups have been retrogressive and decadent, expressive of factorial degeneracy. The ostrich and the entire group to which it belongs, the Ratitae, may be deemed to be one of these at the present time; in this respect they represent probably the most striking group of degenerate vertebrates which could be adduced. Parts of the germ plasm at least must be common to the entire sub-class, and may well be subject to the same senile changes.

The slow, continuous, determinate nature of the changes going on in so many directions in the ostrich is altogether in harmony with the conception of a gradual advent of senility. Somatic cells, as becomes their temporary, individual character, are all too rapid in their approach to old age; whereas germ cells, as becomes their racial significance and value, approach decadence with extreme slowness. If we countenance germinal senility in the ostrich it is however as a senility of the individual factorial constituents of the germ plasm, not of the germ as a whole; the factorial conception of the nature of the organisms would lead us to expect this, though the latter might easily be contemplated. Were any support needed for the hypothesis of genetic factors, the independence of the various retrogressive changes in the ostrich would of itself be most convincing.

It would be an attractive reflection if, in such a case as the ostrich, we could establish that in a general way the order of retrogressive evolution is the reverse of that of progressive evolution. Feathers, scales and claws are indeed recent phylar acquisitions, and the essential vertebrate structures were laid down long before digitate limbs came into being.

On the above conception of the germ plasm of the ostrich as undergoing regular successional changes in various directions, perhaps of the nature of fractionation and loss, it is implied that the changes are in progress among the individuals of the race as a whole; in other words, that the ostrich race presents us with an example of mass mutation. The degradation tendencies within the germ plasm being the same throughout, the changes appear independently in the individual members of the race. In his Presidential Address before the British Association in Australia in 1914 Prof. Bateson expresses himself in the strongest terms against a view of this nature: "Modern research lends not the smallest encouragement or sanction to the view that gradual evolution occurs by the transformation of masses of individuals, though that fancy has fixed itself on popular imagination." It needs however to be borne in mind that in the case of the ostrich we are in a position to observe the changes in representatives from an area continental in its extent, that certain specific characters show there has been no recent intermingling between the members towards the extremes of the area, that the changes are wholly intrinsic and of no appreciable selection value, and that similar retrogressive mutations occur throughout, differing only in the smaller details of the stages reached. The main facts are of an altogether different order from those hitherto specially studied by Mendelians, and may well be expected to influence our views accordingly.

FACTORIAL CHANGES.

Another striking feature in the retrogressive changes going on in the ostrich is the multiplicity of the methods followed, from which it may be inferred that a corresponding diversity of germinal changes is involved. Without the necessary experimental support it may be held that no good purpose will be served by discussion of the latter, especially in view of the warning from Prof. T. H. Morgan as to the danger of interpreting the nature of a germinal change from its expression in the soma. The absence of any likelihood however of carrying out detailed Mendelian experiments with the slow-breeding ostrich emboldens one to attempt to bring the many observations into some sort of line with the principles embodied in the factorial hypothesis, representing as it does the furthest attempt yet made to understand the germinal nature of organisms and the changes which they undergo.

That degeneration has long been in progress in the history of the ostrich, and is in operation at the present day, appears to admit of no question. Evidence has been adduced that many structural parts have already disappeared, while the imperfect and vestigial conditions of others are deemed to be the various mutational stages leading to still further losses, and in the case of the claw and scales experiments have shown that selection can induce and accelerate the losses. These are but the outward manifestation of internal changes going on in the germ plasm. Hitherto studies in mutation have been largely confined to fortuitous changes, and the genetic behaviour of large and conspicuous characters; but with more intensive studies and continued application workers like Prof. Morgan and his Columbia associates have reached mutations so small as to be scarcely appreciable, and have been able to extend their observations to changes in the actual chromosome itself. In addition, most of the variations yet studied have been discontinuous in their nature and apparently unconnected with others, a notable exception being those of Prof. W. E. Castle on hooded rats; but in the ostrich we encounter variations of the first kind along with others which are successional and seem part of a determinate plan. The former may well be due to irregularities in germinal mitosis, but the latter certainly call for consistent factorial changes, generation after generation. So determinate are the changes that we can with every assurance predict what some of the later ones will be. Thus there can be no reasonable doubt that the fourth toe will disappear in time and that the upper-coverts

will be greatly reduced in number, following along lines similar to those of the under-coverts.

The production of a bald head patch in the northern ostrich and the loss of the feathers from the legs of both the northern and southern birds are examples of true mutations in the sense in which the term is employed by de Vries, Bateson and others. They are discontinuous, striking variations, albeit coming late in the chick ontogeny. The head patch has been proved to represent a Mendelian character, dominant in first crosses and segregating in the second. Whatever the degenerative influence may be, whether factorial atrophy, some inhibitory modifying factor, or a separate factor for baldness, it acts fairly suddenly and completely for an entire area. The change from plumage covering to baldness is effected as a single, complete, disconnected, retrogressive act, altogether different from the slow, successional losses among the coverts and remiges of the wings. If however, as Morgan and others would have us do, we regard each factorial change, however small it may be, as giving a unit-character or mutation, then it becomes impossible to draw the line between continuous and discontinuous variation so far as magnitude alone is concerned. The slow, gradual reduction in size of a plume would represent a series of barely perceptible independent mutations, while the sudden, complete losses of feathers from the head and legs would be only mutations on a bigger scale. The nature of the changes might be the same; they would differ only in degree. The fundamental difference would then be that the latter are disconnected changes while the former are successive and cumulative, all in the same direction; the two are isolated detached mutations, the others are part of a continuous scheme.

That fortuitous, disconnected mutations, as contrasted with successive mutations in the same direction, are the main foundation of the Mendelian conception of the evolutionary process is clearly indicated by Morgan in the section, *Chance Mutation and Natural Selection*, in the paper so often referred to, where he says (p. 51): "The mutation process rests its argument for evolution on the view that among the possible changes in the genes, some combinations may happen to produce characters that are better suited to some place in the external world than were the original characters." To mitigate this entire appeal to chance, without introducing into the theory of evolution some sort of directive agent to account for cases of successional changes, he then states a relation between chance and evolution which he conceives to be of fundamental importance: "Starting at any stage, the degree of development

of any character increases the probability of further stages in the same direction....In this sense evolution is more likely to take place along the lines already followed if further advantage is to be found in that direction." It is contended that the facts disclosed by the ostrich show that the principle of sequential, cumulative evolution holds quite apart from the last clause; "advantage" has nothing whatever to do with the changes nor with the direction which they follow.

The claw on the fourth toe is perhaps the simplest, most straightforward example we can select from among the many structural parts which are undergoing degeneration, and discussion of some of the genetic principles involved may well centre around it. It may be allowed that a continuous series of stages can be found between its maximum development and its last trace in the integument before its final disappearance, the smallness of the steps between one stage and the other depending upon the number of claws available, a theoretically large enough number giving a theoretical continuity. But what are the factorial issues involved? Have we a single germinal factor or even group of factors which is undergoing as slow and continuous a weakening, or is it a successive loss of factors each of which has such a small expressive power that apparent continuity prevails? The question can be stated in several other forms: Is the individual factor itself capable of variability, of changing its potency up and down, as it were, in a continuous degree; or are the variations in its expressibility dependent upon a successive loss of the constituent factors of a main factor (multiple allelomorphs); or is the factor itself immutable and degeneration the result of other factorial influences acting upon it, the latter being of a modifying, controlling, or inhibitory nature (multiple modifying factors), and possibly arising as a fractionation of a complex factor?

Discussion of the various aspects of the problem of factorial variability is most acute at the present time. The controversy centres mainly around the work of Prof. W. E. Castle on hooded rats and that of Prof. T. H. Morgan and his associates on the fruit-fly *Drosophila*. The issues are plainly stated by Prof. H. S. Jennings, as follows¹: "Castle finds that in rats he can, by selection, gradually increase or decrease the amount of colour in the coat, passing by continuous stages from one extreme to the other. As to this, he holds two main points:

"1. The change is an actual change in the hereditary characteristics

¹ "Observed changes in Hereditary Characters in relation to Evolution," *Jour. Wash. Acad. Sciences*, Vol. VII. 1917, p. 287.

of the stock; not a mere result of the recombination of Mendelian factors. This is the general and fundamental point at issue.

"2. More specifically, he holds it to be an actual change in a single unit factor; this single factor changes its grade in a continuous and quantitative manner.

"On the other side, the critics of these views maintain that the changes shown are not actual alterations in the hereditary constitution at all, but are mere results of recombinations of Mendelian factors. And specifically, they find a complete explanation of such results as those of Castle in the hypothesis of *multiple modifying factors*."

Prof. Jennings then proceeds to discuss in much detail recent work on colour changes in the eyes of *Drosophila*, most of which are minute differences. These are claimed by the mutationists to be due to the influence of *multiple modifying factors*, by means of which a visible character may be modified in the finest gradations by alterations in diverse parts of the germinal apparatus, and by *multiple allelomorphs* which show that a single unit factor may exist in a great number of grades. "The very facts known for *Drosophila* show that there is nothing to prevent a passage from one extreme to the other by minute changes, just as is held by the palaeontologists and selectionists, although change by large steps occurs also"... "the mutationist thinks of all these numerous grades as after all essentially discontinuous, as a series of steps so minute that the difference between one and the next one is not detectible. His opponent, on the other hand, perhaps thinks of the series as actually continuous. But the difference is not a pragmatistical one; when steps become so minute as to be beyond detection, the question whether they exist becomes metaphysical."

Until we have direct evidence to the contrary the same principles must be held with regard to the smallest step in the degenerative process as to the dropping out of the large individual characters, which we usually think of as mutations. They are all changes held to be effected during the mitotic reconstruction of the zygote, and Morgan has shown that in *Drosophila* each change almost certainly involves a change in a definitely localized part of a chromosome; and with good reason he regards each step, whatever its size, as a unit character or mutation. Though to de Vries a mutation, and to Bateson a discontinuous variation represents usually a large, obvious character, closer analysis shows no reason why the somatic expression of every factorial change, however small its degree, should not be regarded in the same sense. In this case every stage in the diminution of the claw or of a plume would

represent a separate mutation, a distinct unit character, even though not somatically recognizable.

It is barely conceivable that any factorial alteration does not represent some separate, distinct degree of change rather than a continuity. The former may be ever so small, but is still a discrete amount, as in all Morgan's results. On the conception entertained by Castle of changes up and down of the same factor the passage from one stage to the other could be held to be continuous, while on Morgan's conception it would be step-like. Somatically no difference need be apparent between the two, but the underlying conceptions are different and are important from a Mendelian point of view. It is not the degree of somatic change which matters, as Jennings rather implies, but the manner in which it has been factorially effected, and all considerations seem to favour a graded or step-like process as compared with a continuous one. It may of course be argued that a flight of stairs becomes an inclined plane if the steps are made sufficiently small and numerous, and a structure in a successional, degenerative phase may appear to make its descent down a plane, instead of in a graded fashion.

Though a continuous series of stages in the reduction of the claw could be procured yet it does not follow that a like continuity is followed by the factorial changes. They could just as readily be discrete germinal changes, and it is held that the seemingly continuous degeneration of the plumes and digits is of this character. So many influences are concerned in the somatic expression of factors that it becomes no sure guide as to the nature of the factor itself where comparatively small changes are concerned. Changes may be discrete and successive and yet give somatic continuity so far as our observation can extend. On the conception of every somatic change being a step the term *rectigradation* of Prof. H. F. Osborn is peculiarly appropriate compared with that of continuous variation; for the latter has a somatic application only, whereas the former gives the factorial significance in addition. Variations in any definite direction may be continued without being continuous.

So far then as concerns the retrogressive changes in the claw on the fourth toe of the ostrich we may think with Castle, and those who agree with him, that it is due to a continuous retrogressive change in the factor itself; or we may hold with Morgan and his supporters that the change is due to multiple allelomorphs, perhaps of the nature of so many successive losses or changes in the main factor, or to the influence of multiple modifying factors influencing the expressive nature of the claw factor.

Such diverse factorial possibilities for an apparently simple process as a continuous reduction in size of a claw serve to reveal that until experimental mutation can be reduced to simpler terms its results can hardly be used as an instrument for procuring a clear conception as to the factorial changes involved in evolutionary processes generally, and the evolutionist may have to remain content with the somatic expression of his characters by whatever germinal process they may have come to be. The evolutionist would naturally desire to bring his facts into harmony with Mendelian results, and adopt their interpretation, but when any simple change may apparently be due to any one of such a multiplicity of germinal agencies, any assurance of correctness can only be gained through separate studies of the magnitude of those already carried out by Morgan, Castle and others. The recent paper by Prof. Morgan, "Evolution by Mutation," full as it is of warnings as to the dangers of interpreting somatic changes in factorial terms, is sufficient commentary. Probably Morgan is quite justified in his remarks "It is true that when writers have brought forward evidence of continued and progressive change in a character they have not concerned themselves with the analysis of the change in the germ plasm that has brought it about—in fact, in most of these cases the possibility of advance in a principal gene or of advance through modifying genes had not been appreciated or even understood." But when one thinks of how many years of intensive work it has taken to arrive at some of the conclusions, and with material peculiarly well adapted to the purpose, it is small wonder that students of evolution have not been able to do the same. Perhaps it may be replied that it is only the evolution of the germ plasm which counts, that of the soma being merely a product of the germ, and of secondary import. For in the end, evolution is determined by the germ plasm rather than by the soma.

The remarkable variation between the right and left sides of bilateral structures which are in a degenerative phase would appear to be of some significance. All the degenerating structures of the ostrich display it—plumes, scales and claws; the limit of variation being approximately that of the degenerating element or elements for the time being. Thus among the plumes a difference of only one or two is displayed by the two wings, and in any row usually only one or two of the end members are in a degenerative phase; a vestigial claw may be present on the toe on one side and not on the other. If in the same individual an asymmetry of this nature is possible between the two sides produced from the same zygote it engenders reflections as to the uncertainty of the facts of varia-

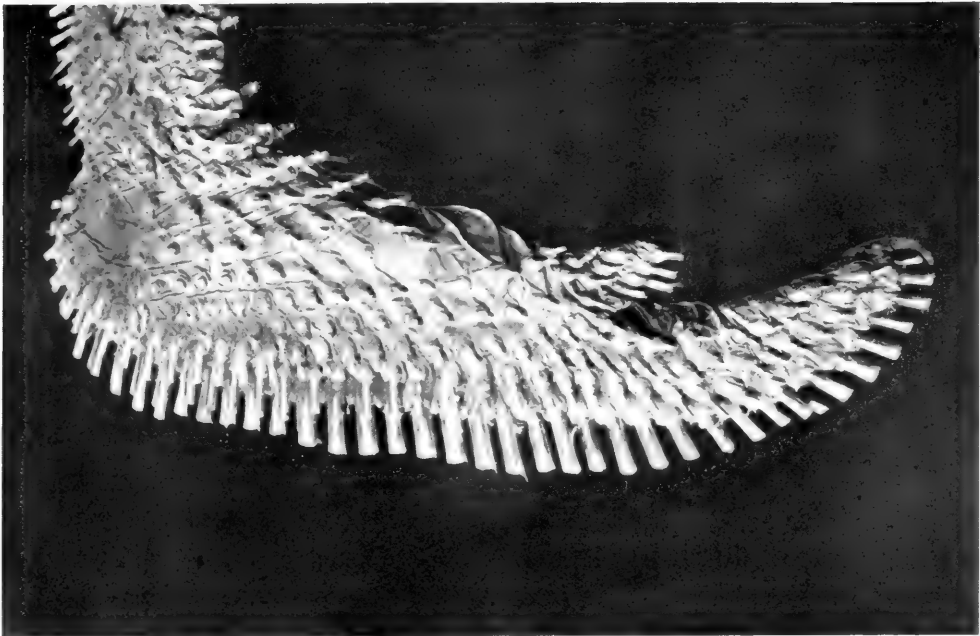


Fig. 1.

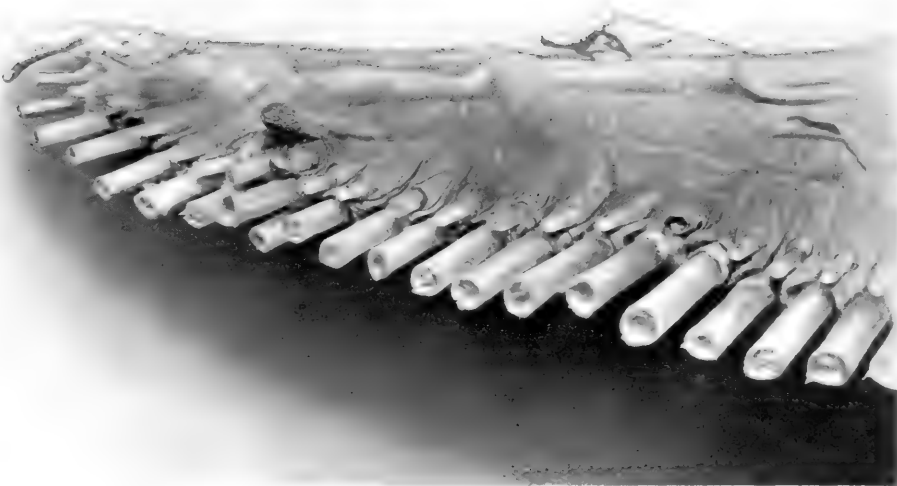


Fig. 2.



Fig. 6.

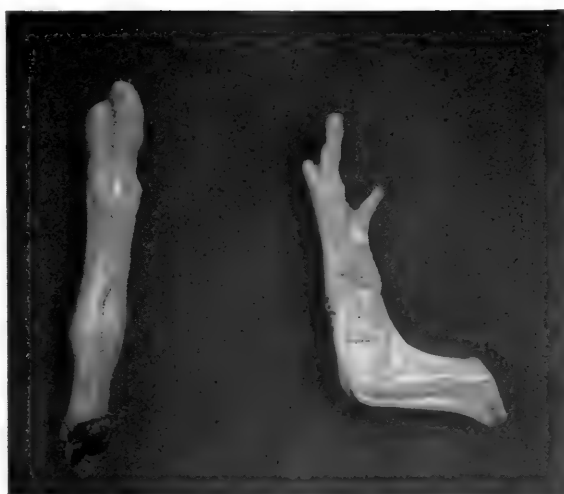


Fig. 5.



Fig. 3.

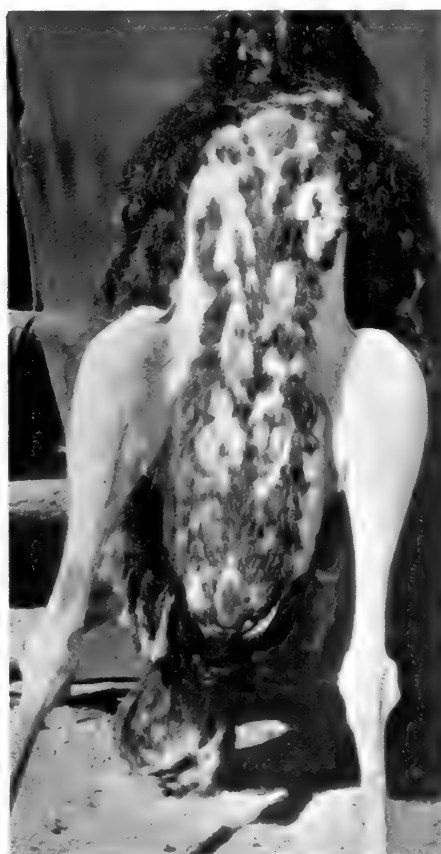


Fig. 4.



tion generally, and also as to the diverse results which may be obtained from the same factors when in a changing state. From a wholly different point of view it fully supports Morgan's contention as to possible discrepancies between somatic expression and factorial condition; it may also enable us to understand how an appearance of somatic continuity can be conferred though the germinal changes are discrete and sequential. At any rate when factors are in a variable state it must create a strong sense of insecurity in the expressions obtained.

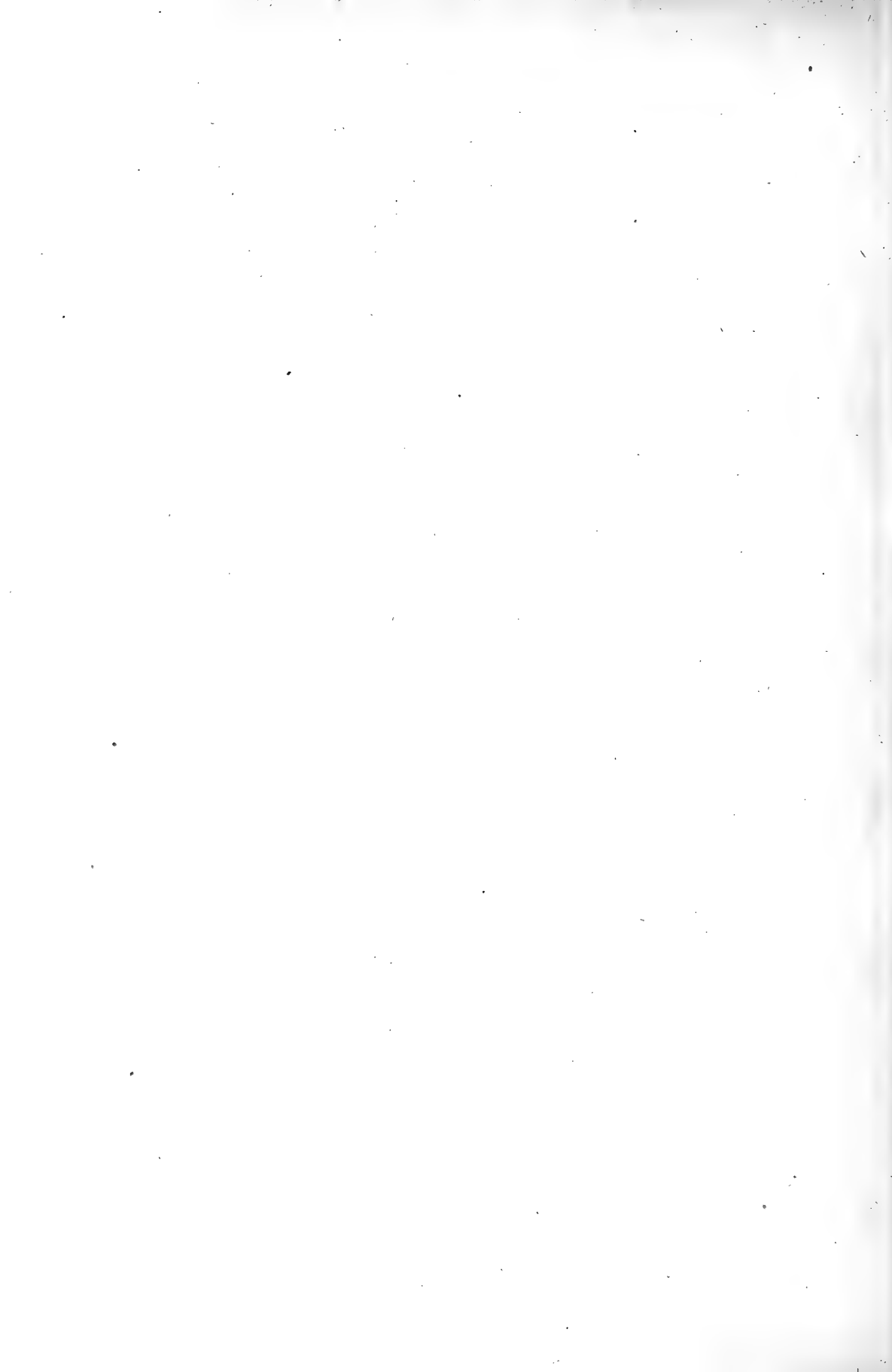
DESCRIPTION OF PLATES.

PLATE V.

- Fig. 1. Outer surface of wing with plumes clipped from quills. South African ostrich.
 Fig. 2. Under surface of terminal part of wing. The bastard wing is just visible above, the second finger with its claw extends to the tip of the wing, while the third finger projects freely from the general surface below, its tip rounded and callous. Near the third finger an irregularity in the row of under-coverts and of wing-quills occurs, the two additional feathers belonging to the third digit.

PLATE VI.

- Fig. 3. Three young ostrich chicks showing upper part of leg covered with natal down.
 Fig. 4. Under surface of ostrich to show the featherless condition of the legs, the lateral apertures, and the sternal and pubic callosities. The pits of the sockets from which the feathers have dropped out can still be seen on the legs, and also one or two persistent feathers. The bird is about eighteen months old and the white ventral feathers are not yet wholly replaced by the black feathers of the adult cock plumage.
 Fig. 5. Wing of embryo chick (left), about fourteen days' incubation, showing the first three digits. The third finger below is much shorter than the second and gradually becomes proportionately smaller.
 Tarsus and foot of embryo chick (right), about fourteen days' incubation. The third and fourth toes are of nearly equal size while the second appears as a slight projection on the left.
 Fig. 6. Tarsus and foot of young chick with scutellation at about same stage as that in Fig. 7 and like *e* in Fig. 8.



GENETICAL STUDIES IN THE MOTHS OF THE
GEOMETRID GENUS *OPORABIA* (*OPORINIA*)
WITH A SPECIAL CONSIDERATION OF MELAN-
ISM IN THE LEPIDOPTERA.

BY J. W. HESLOP HARRISON, D.Sc.

(With 13 Text-figures.)

CONTENTS.

	PAGE
I. Introductory	195
II. Variation—Subspecies—Local Races—Melanism	205
(a) General	205
(b) The evolution of the subspecies <i>O. filigrammaria</i>	209
(c) The evolution of the local races	214
(d) The case of <i>O. dilutata</i> and its subspecies <i>O. christyi</i>	228
(e) The question of melanism	229
III. Breeding Experiments	246
(a) Inheritance of subspecific characters and of those characterising the local races	246
(b) Inheritance within the limits of the local races	249
(c) Inheritance of melanism	249
(d) Sex-linked inheritance	250
(e) Hybridisation experiments	254
(1) Hybrids between <i>O. autumnata</i> and <i>O. filigrammaria</i>	254
(2) Hybrids between <i>O. autumnata</i> and <i>O. dilutata</i>	266
(3) Miscellaneous pairings	275
IV. Summary	278

I. INTRODUCTORY.

THE genus *Oporabia* (now becoming known as *Oporinia*)¹ is a homogeneous genus of the Geometridae, occurring throughout the Holarctic region, but in spite of its enormous range possessing very few species, in which peculiarity it resembles its nearest allies the genera *Venusia* and *Cheimatobia*. From the former the original phase of both *Oporabia*

¹ And in America as *Epirrhita*!

and *Cheimatobia* has been derived; the latter appears to have diverged from their common ancestor at some fairly early stage in its existence. With *Venusia*, owing to the period of its active life cycle, we are not at present interested; in the case of *Cheimatobia* matters are somewhat different, for in opposition to the indications of its apterous and subapterous females, it approaches so closely to *Oporabia* in all its other essential characteristics as to render any investigation in that genus incomplete without their joint genetical possibilities being considered.

In view of this limitation in the number of species included one would have anticipated that the genus would prove, from the standpoint of specific differentiation, an easy subject for study; such has not, however, been the case, as only comparatively recently have the relationships between the forms it includes been understood with any degree of exactitude. As a result of recent researches it can now be confidently stated that the genus comprises two species and two subspecies, the species *Oporabia autumnata* with its subspecies *O. filigrammaria*, and the species *O. dilutata* with *O. christyi*. So chaotic did the variation of the species appear, and so prone were they to vary along parallel lines and to form local races resembling each other, that entomologists, even whilst keeping the subspecies *O. filigrammaria* distinct, consigned the remainder of the forms, comprising two species, one subspecies and countless local races, into one specific dustbin which they labelled *Oporabia dilutata*. Such a procedure would have been impossible had they had the slightest knowledge of the insects not as dried specimens but as living creatures, each with its special life history and specialised habitat. In this lumping they were excelled by Meyrick¹, who in this as in all similar cases cut the knot of his difficulties by lumping all, the very striking *O. filigrammaria* included, under the same specific title. Ignoring the practice of Meyrick as thoroughly unscientific, the action of entomologists in general ended in the severing of two of the most closely allied forms, and the uniting of two of the most physiologically diverse forms in the British Fauna. This was most vividly proved by the almost perfect homology of the chromosomes in the gametogenesis of hybrids between *O. autumnata* and *O. filigrammaria* and its absolute lack in the case of the *dilutata*—*autumnata* hybrids.

For the order which now obtains in the genus three workers are responsible, Prout, Allen and myself; Prout and Allen worked from the standpoint of the systematist and I from that of the geneticist, although necessarily my labours unearthed many facts of great value to systematics.

¹ Meyrick, *British Lepidoptera*, p. 224 (1896).

Thanks to these workers, then, the forms are easily separated on structural characters derived from both sexes but especially from the male. In fact, the forms are most readily differentiated by separating the males and then allowing the females to fall naturally into their own places. The primary division into the two species proper is best based on the genitalia, a prominent hook on the genital claspers marking *O. dilutata* (Fig. 1) and *O. christyi* (Fig. 2), and its absence *O. autumnata* (Fig. 3) and *O. filigrammaria* (Fig. 4). To distinguish *O. dilutata* and *O. christyi* one then examines the sternite of the eighth abdominal segment upon which in this genus there are two chitinous projections known as octavals. If these be approximated the insect is *O. christyi*, but if not it is *O. dilutata*. Similarly, by examining the same structures, *O. autumnata* and *O. filigrammaria* can be discriminated. If the excavation between the octavals be slight we are dealing with *O. autumnata*; if deep *O. filigrammaria*. In this case the smaller number of hairs on the cristae of *filigrammaria* will give abundant confirmatory evidence; in the former none is necessary. Further major and minor characters serving to distinguish all four forms in both sexes are indicated in Tables I and II below, and as far as the genitalia are concerned illustrated on Figs. 1, 2, 3, 4; for the sake of comparison and to emphasise the relationship between the two genera *Oporabia* and *Cheimatobia* the genitalia of *Cheimatobia brumata* and *C. boreata* are supplied on Figs. 5 and 6.

The life histories of all of these insects follow the same general course, all four forms of *Oporabia* and the two species of *Cheimatobia* hibernating as ova, which hatch as the trees leaf in spring. The larvae feed up exceedingly rapidly in May and early June. When they emerge from the egg they are rather slim in build and of a brown colour, very dark in *autumnata* and *filigrammaria* but lighter in *dilutata* and *christyi*. When adult they are stout and in colour green, of shades and markings varying with the species and subspecies; exact details of the specific and other differences both in ova and larvae are supplied in Tables I and II. As the larvae attain their full growth they bury themselves in the loose debris at the base of the trees and shrubs utilised as food plants and spin small compact oval cocoons constructed, in the more external layers, of fragments of earth, leaves, moss and so on cemented by silken threads, and on the inside of a waterproof coating of pure silk. In these they pupate and remain in this condition until the cooler days of the last weeks of August, or of September and October call the moths forth, the exact period of this event depending on the species, subspecies and local races.

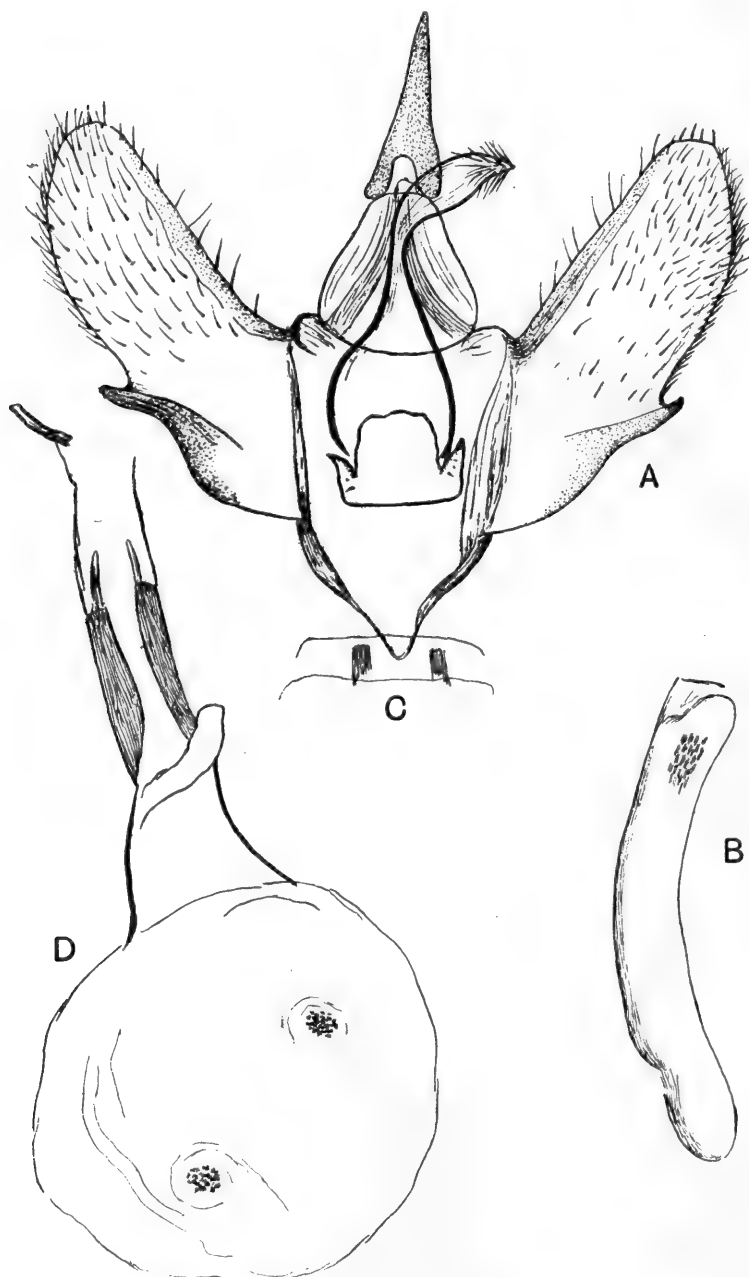


Fig. 1. The genitalia of both sexes of *O. dilutata*.

A = the male genitalia without aedeagus. B = Aedeagus. C = Octavals.
D = The female genitalia.

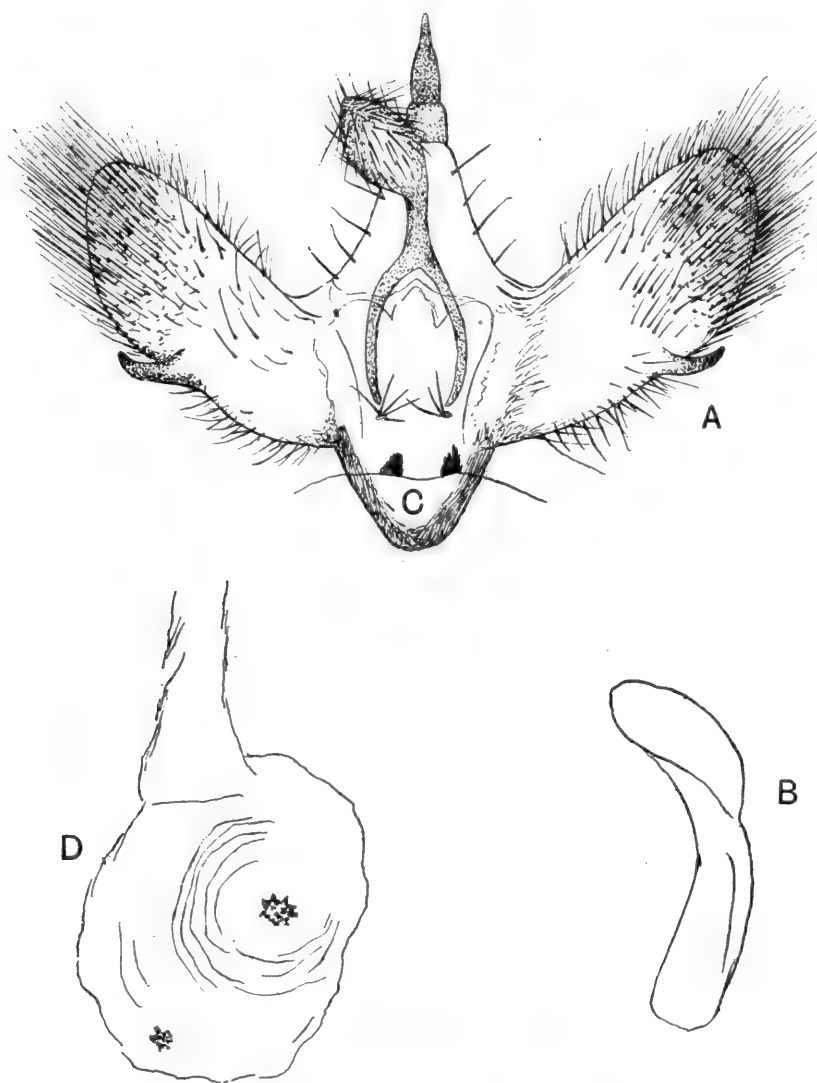


Fig. 2. The genitalia of *O. christyi*.

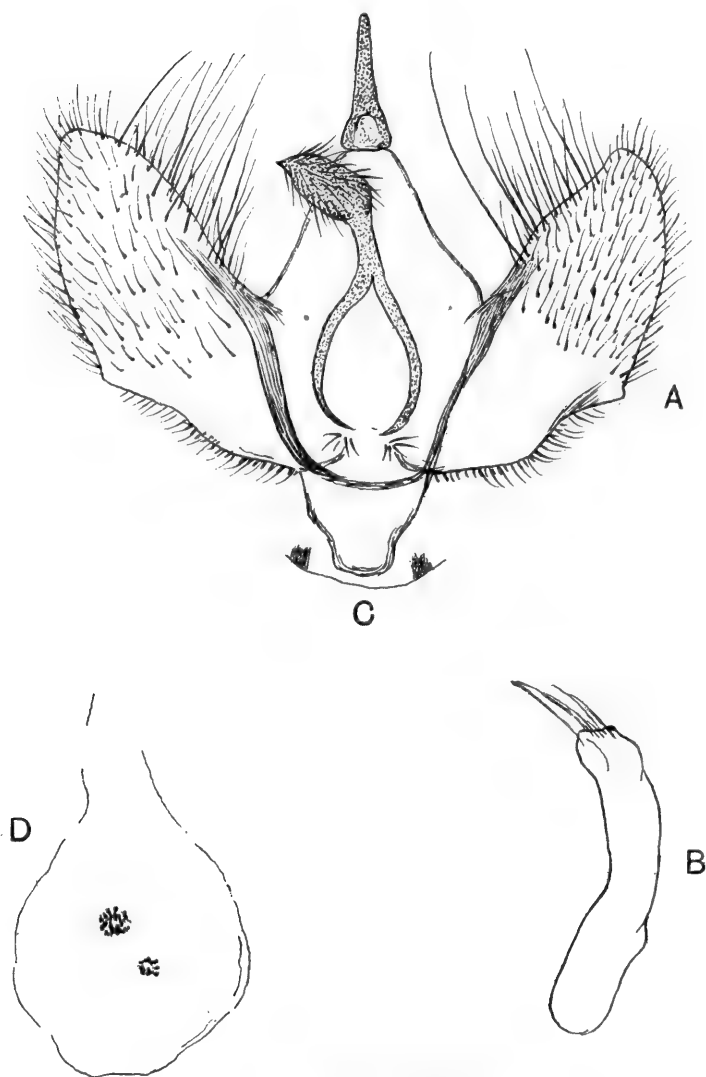


Fig. 3. The genitalia of *O. autumnata*.

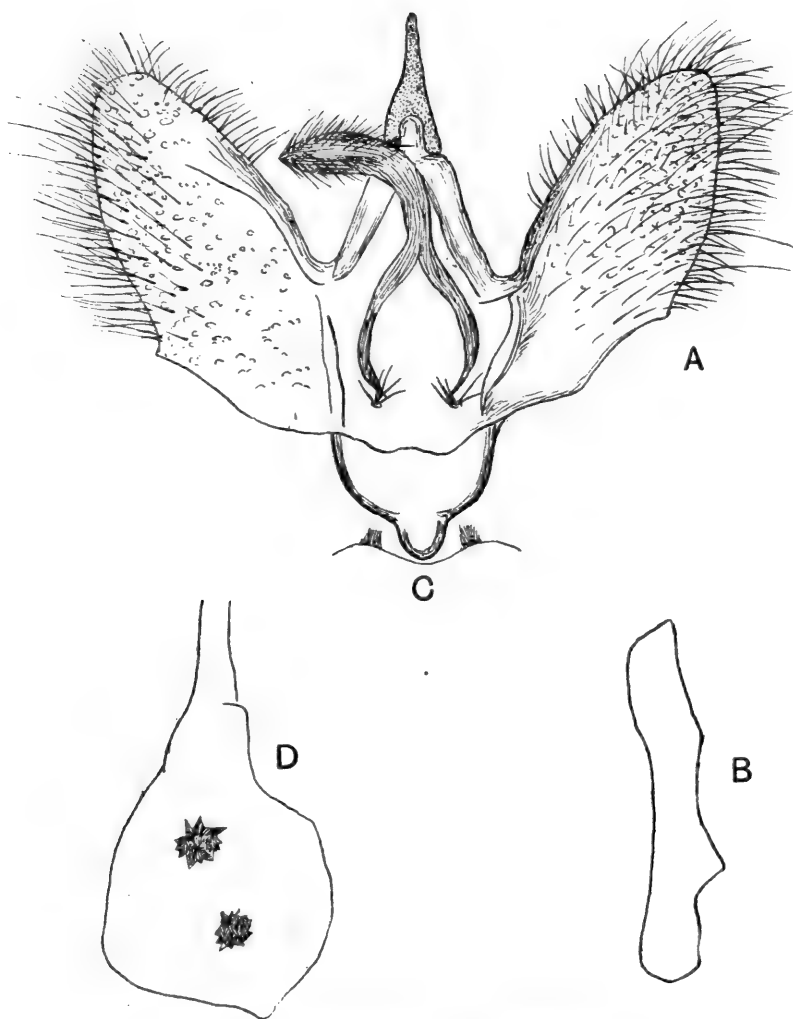


Fig. 4. The genitalia of *O. filigrammaria*.

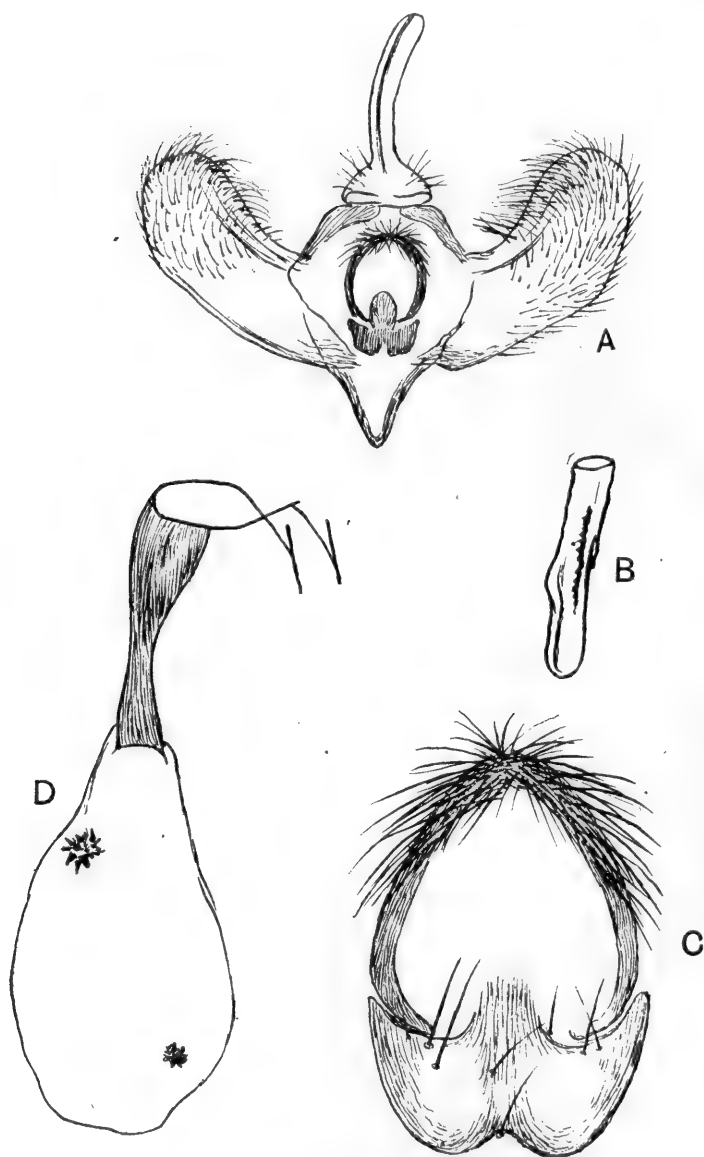


Fig. 5. The genitalia of *C. brumata*.

A=Male genitalia without aedeagus. B=Aedeagus. C=Labides. D=Female genitalia.

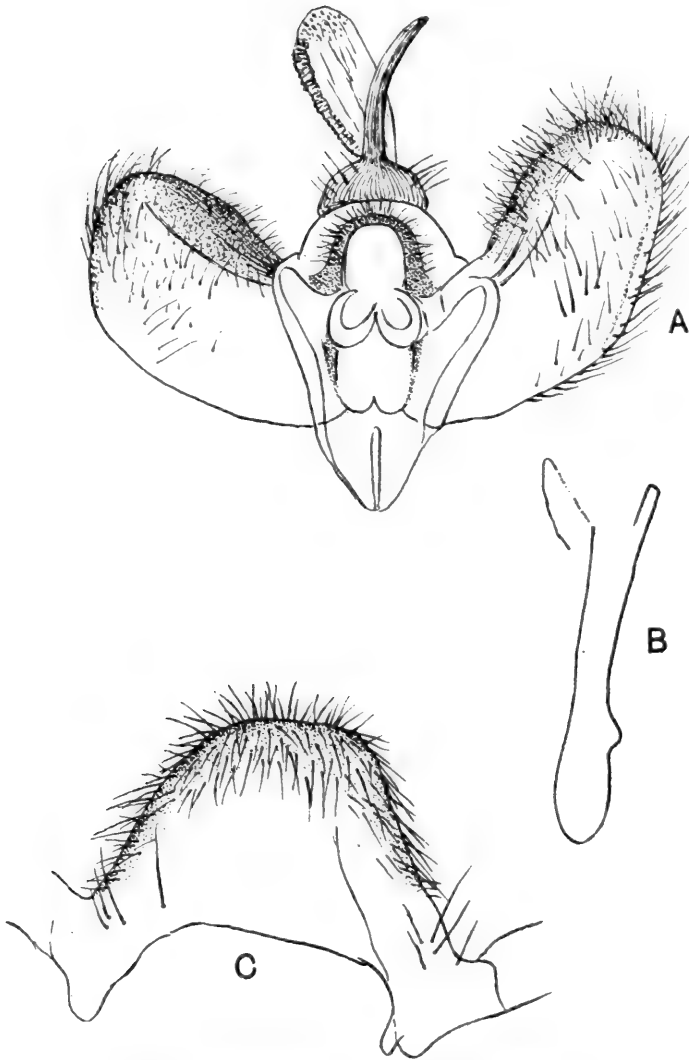


Fig. 6. The genitalia of *C. boreata*. Male only.

The imagines emerge in the afternoon and remain during the rest of their first day at the base of the tree boles and amongst the herbage. After the first evening's flight they rest higher up, often enough very conspicuously on tree trunks, but sometimes on smaller twigs and amongst leaves. Both sexes, the female in particular, delight to sit on the smaller twigs with their wings encircling them. *O. filigrammaria* in some of these respects necessarily differs; both sexes sit on heather twigs, but if rocks are available they may be seen dotted about them in considerable numbers. In all cases the insects are easily startled or dislodged, the males flying high and far and the females but a short distance; in fact, not uncommonly the female feigns death and drops amongst the herbage.

The eggs are laid at the base of buds either on the bud itself, between it and the stem, or under tiny curled pieces of bark where they remain sheltered from all the vicissitudes of winter.

It was my good fortune to discover all of the forms referred to above in my own immediate neighbourhood and therefore within easy reach for study. I at once commenced to experiment with them, and very early indeed I discovered their interspecific fertility and, in certain cases, that of the hybrids of the F_1 generation. These latter observations, coupled with the discovery of the constancy of the several local races evolved from *O. autumnata* and *O. dilutata*, formed a combination so attractive to a geneticist that I determined to avail myself of the opportunities thus offered and to submit the whole of the phenomena presented to more intense study. In doing so I propounded for solution the following problems:

(1) Seeing that *O. autumnata* and *O. filigrammaria* are perfectly fertile when crossed, and since the F_1 hybrid generation is likewise fertile when paired *inter se* or when back-crossed with either parent species, is the type of inheritance shown explicable on strictly Mendelian lines?

(2) What light do the hybrids between *O. autumnata* and *O. dilutata* throw on the same problem?

(3) Further, since *O. autumnata* and *O. filigrammaria* differ in mean size in both sexes, what information can be gleaned from their study as to the inheritance of size?

(4) How do these same two forms maintain their distinctness even when free to cross through their colonisation of the same or adjacent areas?

(5) Do the differences between the various local races, as well as

those between *O. autumnata* and *O. filigrammaria* and between *O. dilutata* and *O. christyi*, invariably reappear in the offspring?

(6) If so, is this actually due to heredity and therefore germinal, or is it ontogenetic, i.e. due to like environmental impulses acting anew on each individual of the successive generations to produce like effects?

(7) If actually germinal, how do the differences arise and how are they fixed; are we concerned with "germinal" mutation or with acquired characters depending on the prolonged action of environmental agencies and their final registration in the germ cells with subsequent inheritance?

(8) Are the current explanations of melanism in the Lepidoptera in harmony with the facts; and what evidence has the genus *Oporabia* to offer on the subject?

(9) In all of the problems enunciated how far has "natural" selection played its part?

By a perusal of the above questions it will be seen that I undertook the work without allowing my natural bias against the Lamarckian position to prejudge the case; what answers I obtained to them will be discovered in the succeeding pages.

II. VARIATION—SUBSPECIES—LOCAL RACES—MELANISM.

(a) General.

In the introductory remarks I indicated that the genus *Oporabia* included two species and two subspecies, *O. autumnata* with its subspecies *O. filigrammaria*, and *O. dilutata* with *O. christyi*. It now behoves us therefore to consider the exact relationship, if such consideration is possible, between these pairs of forms, and incidentally to assure ourselves of the value to be attached to the term subspecies.

In my earlier work I was always sceptical of the existence of such things as subspecies and shrank from using the word. Prolonged study of the present genus has considerably altered my views in this respect, so that I have come to recognise the existence of such entities as exceedingly probable, and in any case the use of the word as justified. In *Oporabia* the two subspecies recognised, when assessed at their true value, do not depart very widely from the Jordanian concept of the "little species," or, as I have usually called it, the "microgene." Furthermore, the probable course of development of *Oporabia filigrammaria* gives us some hint that such subspecies or microgenes may have arisen by a long period of isolation not in itself of sufficient duration or of such a type as to allow of divergences of full, unchallengeable, specific value.

Any exact or even approximate definition of what constitutes a subspecies is, however, an enormously complicated matter, rendered the more so by the uncertainty as to what a species itself really is.

If we compare *O. autumnata* and *O. filigrammaria* we find that in important structural characters they differ not at all; moreover, they are perfectly fertile when mated together. Nevertheless, when one actually tabulates the differences (as I have done below, as well as those between *O. dilutata* and *O. christyi*) we find that the insects fail to agree in many minor points both in structure and in life history. In addition each has the power of setting up its own peculiar local races—races each one of which is characteristic of its own locality, but in *filigrammaria* never altering its fundamental details to show that its degree of differential evolution passes from that of its fellows elsewhere toward the *O. autumnata* with which it is phylogenetically connected: and the same remarks apply to *O. dilutata* and *O. christyi*. Probably the best view to assume concerning the subspecies is to regard the term as covering those cases in which we have groups, sufficiently differentiated from those pronounced by systematists as good species for one to regard them as possible species, but upon which one is forced to suspend one's judgment, either because of a lack of clarity as to what a species is, or because one cannot gain sufficient of the essential details upon which to found a decision free from any tendency to waver. The very great difficulties in the way of gaining the necessary decisive facts are emphatically shown when the perfect fertility between *O. autumnata* and *O. filigrammaria* and between *O. dilutata* and *O. christyi* is contrasted with the puzzling behaviour in that respect of certain forms of the genus *Orgyia* which I can only regard as subspecies; the matured judgment of years of study will not permit me to treat them as being of specific rank. These are those subspecies of *Orgyia antiqua* known as *O. badia*, found in Pacific North America, and *O. nova* in North America east of the Rocky Mountains. In every character the two American forms are widest apart, *O. badia* being almost unrecognisable as the same species as *O. nova* when one examines larva, pupa and imago. *O. antiqua* from Europe on the contrary is excessively near to *O. nova* and thus differs greatly from *O. badia*.

Nevertheless, whilst the two American forms, when crossed together, are reciprocally fertile and continue so as long as one cares to pursue the experiment, when one crosses either of the American forms with the European the cross is only fertile when the European insect provides the female, but, as before, the brood so produced can be inbred in-

definitely. The case of *Lymantria dispar* and its subspecies *japonica* produces evidence of the same value.

Local races are in quite another category and no genus is more prone to their development than *Oporabia*, the insects in nearly every habitat displaying local facies. These races are clearly brought into being in response to environmental forces of varying incidence which are not powerful enough to push the insect on to an evolutionary plane palpably higher than that of its relatives in other stations. The differences exhibited may be in any feature—markings, habit, period, etc., each and all being involved,—but they never attain to differences in structure even of the low order existing between *O. autumnata* and *O. filigrammaria*; there is never any difficulty in assigning the insects to the species to which they belong. So near do some genuine local races approach what systematists pronounce the type that only very careful biometrical study can demonstrate that difference in modal condition indicative of the local race.

In general, however, if a sufficiently great number of individuals be assembled from any given area (restricted it may be in some cases but of considerable extent in others) and placed alongside a similar assemblage from another point it will instantly be impressed upon one that the two sets are not alike. Sometimes each individual in the one differs from each in the other by some more or less tangible characters or degree of development of those characters, and sometimes only the majority of individuals show this difference; nevertheless it exists, and such diverse sets can only be regarded as local races.

In *Oporabia*, whilst the differentiation of subspecies and local races is fairly easy, great difficulty lies in the way of assigning any given specimen in the absence of exact data to its correct group, so great is the degree of fluctuating variation exhibited in wing markings. No species or subspecies is free from it, although *O. christyi* is least affected, *O. filigrammaria* being next in order. But, let it be noted, even they can cover the whole range. In all the ground colour can vary from almost pure white to black; the markings in all may be absent, undecided, scattered, condensed into bands, heavy or light and so on with every possible combination of these and the ground colour; even the discoidal point achieves the apparently impossible by displaying great variability.

Haphazard as all this seems, it is in reality quite regular; indeed so regular is it that all four forms possess parallel variations. So obvious was this that when once I knew of the existence of the Carpet-

banded¹ aberration of *O. dilutata* I predicted the occurrence of similar *autumnata* and *filigrammaria* forms and these I have since discovered. A banded *O. christyi* has not yet appeared but it will turn up.

This ordered condition of the seemingly irregular, the appearance of so many wonderful aberrational forms and aberrations in parallel series throughout the species and subspecies of the group is so striking as to suggest that Eimer's theory of Orthogenesis² has some foundation in

TABLE I.

Summary of characters differentiating O. autumnata and O. filigrammaria.

		<i>Oporabia autumnata</i>	<i>O. filigrammaria</i>
Ova...	Description	Salmon pink; pitted, ridges marked but pits shallow	Much the same; perhaps slightly larger and colour duller
„	Hatch	Date varies with season	Always much earlier
Larvae	Description	Apple-green varying slightly in intensity in the local races	Blackish green, mottled appearance; head smoky, smaller
„	Food	Betula, Alnus, Larix, Pinus	Calluna, Erica, Vaccinium
„	Pupate	In typical form late, in larch feeding form earlier	Always preceding any true autumnata form
Imagines	Emerge	In typical form early October to November; in larchwood form September 16th to September 30th	August 20th—September 20th
„	Markings and size	Ground often white, varies to grey; may be melanic; markings generally distinct but feeble and suffused in larch form; female rather large	Colour similar; markings and bars heavier and clearer especially subterminal bar and on hindwings; glossier; smaller; wings narrower; female smaller in proportion to male
Male genitalia	Octavals ³	Not large, widely apart; depression between shallow	Nearer; depression deeper
„	Cristae ⁴	About 19	About 7
„	Labides ⁵	Head wide; vestiture thin	Head narrow
Female genitalia	Signa ⁶ of Bursa copulatrix	Small and scobinate	Larger
Chromosome Number	Haploid	38	37
	Diploid	76	74

¹ That is possessing a complete central band like *Melanthia* (*Larentia*) *ocellata*, *Melanippe* (*Larentia*) *rivata* and *M. (L.) sociata*.

² Eimer, *Orthogenesis d. Schmetterlinge*, Leipzig (1897).

³ Chitinous projections on 8th sternite.

⁴ Patch of stiff hairs on juxta.

⁵ Pair of rods rising from the chitinous bar extending from the costae of the valves; fused and ending in a hairy head in these species.

⁶ Chitinous armature of bursa copulatrix.

actual truth. Furthermore, these phenomena afford, with other similar cases in many genera like *Lycaena* and *Taeniocampa*, some degree of proof of the principle.

TABLE II.

Summary of characters differentiating Oporabia dilutata and O. christyi.

		<i>Oporabia dilutata</i>	<i>O. christyi</i>
Ova ...	Description	Glossy, ruby in colour, pitting feeble	A little smaller, glossier
"	Hatch ...	Varies ...	The same
Larvae ...	Description	Green, often purple marked	The same
"	Food ...	Quercus, Crataegus, Acer, etc.	Betula, etc.
Imagines ...	Emerge ...	October ...	The same
"	Markings, size, etc.	Ground very variable; discoidal point large; angle of elbowed line rounded; sweeps toward discoidal point	Ground whitish generally; discoidal point small; angle of elbowed line a right angle as in autumnata
		Same size as autumnata	Insect a little smaller
Male genitalia	Octavals ...	Far apart ...	Very near
"	Labides ...	Head narrow ...	Head wide
"	Valves ...	Spined externally ...	Shorter; spine more hooked
Chromosome Number	Haploid ...	30 ...	?
	Diploid ...	60 ...	?

(b) *The Evolution of the Subspecies Oporabia filigrammaria.*

Evolved as we have seen from *Venusia cambrica*, whose life history was interwoven inextricably with that of the northern tree *Pyrus aucuparia*, *Oporabia autumnata* accepted birch as a food and was thus enabled to colonise much of the Boreal circumpolar continent of Mid Tertiary times, just as had many of its contemporaries like *Lycia hirtaria* and *Poecilopsis lapponaria*. So like that of *P. lapponaria*, *Nyssia zonaria*, *Anthrocera purpuralis*, *A. achilleae* and *Platyptilia tessaradactyla* are many points of its history that, in view of my detailed treatment of the Bistoninae elsewhere¹, no repetition is necessary here.

Only one fact must be singled out, and that is, that as with the last mentioned five insects, the climax of glacial conditions saw the insect in Europe split into two widely separated colonies, one located in South Eastern Europe and the other in stations far to the west of the British Islands of today.

With these preliminaries we have reached a stage at which the modern history of what now represents the original *O. autumnata* may be regarded as commencing.

¹ Harrison, "The Geographical Distribution of the Moths of the Geometrid Subfamily Bistoninae," *Naturalist*, pp. 164—166, 194—198, 273—278, 377—382 (1916).

One cannot too strongly insist on the enormous environmental differences between the two European sections. That to the east was confined to forests restricted by the barrier of the ice to a dry and rigorous continental climate, whilst the western division existed on low lying shores and islands exposed to a moist oceanic climate modified by the presence of the ice. For thousands of years they persisted thus. Save for oscillations in habitat depending primarily on temporary ameliorations in the climate, the continental colony had to suffer but little change in food. All known inter-glacial deposits yield birch in one form or other; nor is alder lacking. Consequently, for all this period the conditions under which it lived were not altered substantially, with the result that it underwent no significant change in form or habit. Far different, however, was it with that marooned, as it were, on the then far flung shores of Western Europe; to understand fully how different, let us pause to glance for a moment at the vicissitudes of the moorland vegetation in the British area in late pre-glacial and early glacial times.

Heather (*Calluna vulgaris*), a Pliocene product of Ericaceous type, very soon after its evolution on western lands long since submerged by the Atlantic rollers, had united with its more southern brethren of the genus *Erica*, its more robust northern relatives of the genus *Vaccinium* and many other ericetal plants of northern predilections like *Betula*, *Salix* and *Empetrum*, to form an association favouring certain soils. So successful were such communities that the oncome of the glacial period saw them in firm possession of all suitable habitats in the British Islands.

But as the ice sheets crept westward, exposure to steady Atlantic gales, coupled with chilly breezes from the ice-bound land for countless years, had its effect on these societies. Gradually the birch was eliminated, not only through the influence of the winds but also by the incessant fretting of the land by the ocean waves, producing low treeless coasts and islands not unlike what we observe in the Hebrides and Falkland Islands today. Upon such, typical low-growing ericetal vegetation alone flourished. By degrees, with the vanishing of the birch, *Oporabia autumnata* transferred its attentions to *Calluna*. Now, instead of feeding on a leafy deciduous tree in comparative shelter, it fed openly on a low-growing evergreen shrub subject to climatic conditions strange in ways other than those depending on the probably warm and short glacial summer.

Aestivating as it did in the pupal condition, and its emergence as imago in late summer being correlated with the development of the

pupae in response to an exposure to a period of diminishing daily temperatures, it is clear that individuals derived from early hatching ova and from more rapidly feeding up larvae would tend to be exposed earliest to this optimum state of falling temperature and thus would emerge earlier. On the contrary, individuals exhibiting retarded development would tend to emerge so late that the rapid appearance of early winters would destroy them before they could secure the perpetuation of their species. In this fashion, by natural selection, a race provided with early hatching ova and pupae would be built up; *Calluna*, not being a deciduous shrub, would by that very fact favour early emergence and assist this development.

Parallel with the acceptance of a *Calluna* diet another change, probably connected with the difference in nutritive value of *Calluna* and *Betula* to *Oporabia*, occurred, and that was size diminution—a phenomenon that always manifests itself when larvae feed on foliage less nourishing than their own special food and as happened with *O. autumnata* larvae experimentally transferred from birch to heather. But its germinal fixation as well as that of the diet habit itself as a hallmark of the race is another matter. Are we dealing with genuine Lamarckian effects or with a Weismannian case of parallel selection? Are insects whose germinal composition induces that small size which would of necessity demand a shorter larval period than the normal being selected with those undergoing selection because of their speedy feeding up on other grounds? Probably the bulk of present day opinion would favour the Weismannian view but personally I am far from ruling that of the Lamarckian out of court.

The repeated failures in experiments projected to test the validity of the inheritance of acquired characters savour too much of attempting to determine all that occurs in a long express train whilst one compartment flashes by. Besides, one must attach some value to evolutionary experiments in bacteriology even if Sumner's¹ results with mice and Kammerer's² with amphibia are to be neglected. It seems to me that the experience of a hundred thousand years may sooner or later be indelibly impressed on the germ plasm of the race.

¹ Sumner, "The Appearance in the Offspring of Artificially Produced Parental Modifications," *Am. Nat.* Vol. XLIV. (1910). Sumner, "Some Effects of Temperature upon Young Mice and the Persistence of Such Effects in a Subsequent Generation," *Am. Nat.* Vol. XLV. (1911).

² Kammerer, "Direkt induzierte Farbanpassungen und deren Vererbung," *Zeit. f. ind. Abst. u. Vererb.* Vol. IV. (1911). Also similar papers.

Another explanation of the earlier appearance is, however, possible. This *Calluna*-feeding *autumnata* which we will hereafter call *filigrammaria* differs in several minor structural points—cristae, octavals, intensity of wing markings—as well as one major point, the loss of a single chromosome. Now this latter divergence must be mutational in origin; one cannot conceive such a fundamental difference arising by selection. It is therefore possible enough that the change in the life history is intimately bound up with these differences in size and other points, and that all arise from the mutation involving the loss of a chromosome, each tending to the conservation of the race and therefore in the end characteristic of it.

The same might be suggested as an explanation of the curiously significant colour scheme of the larva, both in respect to the actual colours and to its pattern. To this view, however, one great objection can be advanced; they approach too unmistakably to those obtaining in the larvae of other heather feeders like *Anarta myrtilli*, *Agrotis agathina*, *Eupithecia nanata*, and the case is too closely parallel to that of all *Pinus* feeders, whether lepidopterous or hymenopterous, for the matter to be one of a chance mutation. As is generally recognised, mutations take place in all directions from the modal condition and their precise course is a matter of chance; therefore to explain the convergence of the larval designs and colours in all the above ericetal lepidoptera, appertaining to widely separated groups, on such a basis seems too far fetched. We seem rather to be dealing with characters evolved in direct response to environmental forces, like those in Poulton's *Amphidasys betularia*, and fixed by their long continued incidence, aided perhaps by natural selection.

To sum up, attaching due weight to all of the observed facts, chromosome difference included, and considering their origin dispassionately¹, in my opinion the divergences from type *autumnata* are best explained as being brought about by stimuli of the environment, and thus more readily explicable on Lamarckian grounds.

Favourable inter-glacial interludes then saw the subspecies in its present-day guise ready to seize any opportunity of advance, and the waning of the ice, with the exposure of its huge deposits of glacial drift, gave it unique scope for doing so, so readily does *Calluna* invade such ground. But advance was bound to be northward; thus the incipient moorlands along the then common western shores of Scotland and Ireland would be first colonised, followed, as the ice yielded still further and con-

¹ I commenced these studies from a pronounced anti-Lamarckian standpoint.

ditions approached the normal, by gradual infiltration inland until all the heather clad hills of later glacial times were held. Then a slowing-up would intervene, for invading northern forms which had utilised the Scandinavian route as an inter-glacial means of escape from south-eastern Europe would act as a check until recurring glacial conditions restricted the movements of both groups.

Finally the ice vanished and ground capable of colonisation was once more open and to some extent regained, but once again slowly, for we must not forget that *Calluna* is a plant of south-western origin and was passing from more into less favourable areas, and this tardiness kept back the insect, with the result that before the continent was attained the British Islands had come into being.

Advance south-eastward, likewise, was slow; in that direction dense masses of migrating southern forms blocked the way until at length when equilibrium was set up Great Britain was separated from Ireland and the western islands from both; thus the insect was in possession of habitats closely approximating to those it holds today; if one thing is more certain than any other concerning British vegetation it is that the bulk of our heather clad moorlands with their peculiar plant formations are more primitive, i.e. are in a more natural state, than any others.

Accustomed as *O. filigrammaria* had become to a diet of ericaceous plants during the ice age, it had not lost the faculty of feeding up easily and satisfactorily on the *Betula*, *Alnus* and other plants of its earlier days. Yet despite this, even in the birch-heather associations of moorland slopes, the female instinctively lays its eggs on *Calluna* or *Erica*, the birch being entirely neglected. Year after year I have beaten birches in such associations—even seedling trees not so tall as the surrounding heather—on a moor where both type *O. autumnata* and the subspecies *O. filigrammaria* occur, and never has the birch produced *filigrammaria* nor the heather *autumnata*. And this is the more wonderful when one considers the ease with which the confined insect oviposits in chip boxes, on muslin threads, in cotton wool and the like, and on trees when in the semicaptivity of a roomy muslin cage. How has this instinctive choice of *Calluna* and *Erica* become engrained in the germ plasm? It seems very unlikely that it is mutational, and impossible that it should be selectional when due regard is paid to the facts outlined above; once again the evidence strongly urges one to the Lamarckian view that long years of compulsory oviposition on these plants, whether because they were ericaceous or simply because they were low-growing or because of

similar circumstances, have so affected the organism that the habit has been impressed germinally.

(c) *The Evolution of the Local Races.*

Our task now is to trace the sequence of more recent changes in the main species—changes much less profound than those just examined but still, from a genetical standpoint, equally important.

Prior to doing so we must outline the movements of the main body of the species penned up, in all probability, on birch clad plains of Eastern Europe. As I have pictured elsewhere¹, almost certainly all plants and animals of northern proclivities regained our islands from their glacial retreat *via* Scandinavia in warmer inter-glacial times. Amongst these were birches, the remains of which occur so freely in inter-glacial peat deposits both on the land of today and that submerged long ago beneath the Baltic and North Seas. Accompanying the birch would be its insect guests of boreal type, from which we see that *O. autumnata* very early reached our shores; so early indeed was it that like many forms it reached Ireland and the north and west of England ere separation took place.

Having thus very briefly reconstructed the course of *autumnata* to its present range we shall, henceforth, confine ourselves more or less to its movements in the Cleveland district of Yorkshire, the scene of our studies in the evolution of local races.

To equip ourselves for such an enquiry our first object must be the attainment of a broad view of the post-glacial changes in moorland life. The moorlands, here, are of pre-glacial origin and in places like Rosedale have never been over-ridden by ice, from which it appears not impossible that life persisted on the driftless area throughout glacial times. Still we must be careful to notice that, exactly as in Greenland today, whilst *Calluna* may have survived there one would hardly expect such trees as birch and alder to persist. Therefore, for all practical purposes, in considering the post-glacial history of *O. autumnata* we may commence our researches with the recolonisation of the whole country in the equable climate immediately succeeding the great ice age, when the Arctic Flora, in itself decidedly of ericetal tendencies and thus providing a splendid nucleus for moorland plant communities, gradually yielded under the influence of more genial and drier days. Of the condition of the moors then we have ample evidence in the peat beds. Favoured by the cli-

¹ Harrison, "The Geographical Distribution of *Dimorpha versicolora* and what it suggests," *Ent. Mo. Mag.* May, 1916.

mate, far and wide, on dale and hill alike, the moors supported huge birch forests relieved along the streams by alders and giving place to oak and mixed woods nearer sea level. Naturally, its fate being linked up with birch and alder, *O. autumnata* would be equally widespread.

But this dry, warm epoch did not last. A steadily increasing rainfall stimulated the ever greedy *Sphagnum* bogs, and working down from badly drained moors through slacks and gills the developing peat slowly engulfed the birches, as even a casual glance at the stacks of newly cut peat on the remoter moors will reveal, as well as chance exposures elsewhere. Finally all birches, except those growing in ravines supporting a briskly moving stream and those on well drained slopes lower down the dales, were exterminated. Much later even the latter woodlands were destroyed in satisfying the demands of the mediaeval (if not earlier) exploiter of the outcrops of ironstone.

Thus under diverse influences the area under birch was reduced to minute proportions, and on the whole of the northern slopes, including the Eston outlier, almost exterminated, as a glance at those portions of Jeffery's 1772 map of Yorkshire given on Figs. 7 and 8 will show. Neither on Eston Moor nor in Lonsdale is any considerable woodland depicted as existing. And the accuracy of the map cannot be impugned, so correctly are the old established oak-ash woods on the north-east slopes of Eston Moor, Airyholme Wood and Easby Wood indicated; these still flourish just where Jeffery places them.

Such a fate as befell the birches would inevitably have overtaken the insect had not the close botanical relationship of birch and alder always enabled it to use the latter tree as food. And these facts, fortunately enough, put us in the position of being able to assert that toward the close of the eighteenth century, when the period of afforestation set in, the northern colonies of *O. autumnata* were confined to alders lining the various beck sides and those surrounding certain moorland springs and pools, with the possible addition of stray birches. In particular those on the northern outlier known indifferently as Eston or Barnaby Moor were so limited.

A dozen years or so after the publication of Jeffery's map the first attempts at moorland reclamation were made in Kildale and Lonsdale and on Kempswithen by Sir Charles Turner. In Kildale much of the land so reclaimed was destined for agricultural purposes, but in Lonsdale the efforts were directed towards the establishment of plantations including both deciduous and coniferous trees, the upper slopes receiving Scotch fir and larch and the lower oak.

But the afforesting was carried out in a thoroughly unscientific manner, the fatuous planting of oaks, etc., directly on the heather ending in failure so disastrous that not a dozen have survived to the present day. The failure of the projected oakwood was recognised very early and pointed out by Tuke¹ in 1800.

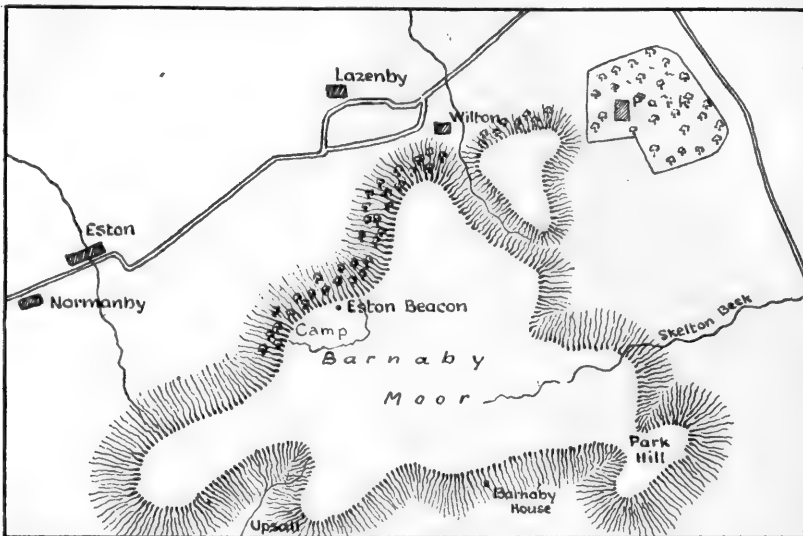


Fig. 7. Eston Moor from Jeffery's 1772 map of Yorkshire.

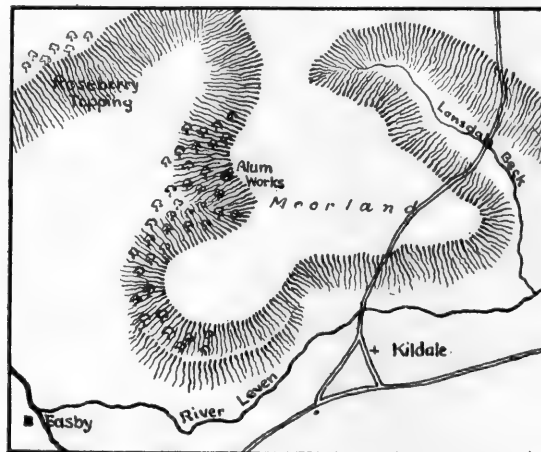


Fig. 8. Lonsdale and Kildale from Jeffery's 1772 map of Yorkshire.

¹ Tuke, *General View of the Agriculture of the North Riding of Yorkshire* (1800).

Subsequently the blanks have been filled, in part artificially with larch, spruce and alder, and in part naturally with mountain ash and alder so that the lower portion of the wood may be classed as mixed larch and alder with the latter predominating (amongst which occur old Scotch firs and poplars), whilst the upper produces spruce and Scotch firs with occasional birches in the open spaces near the hill crests. Mountain ashes occur sporadically everywhere. Also as shown on the map on Fig. 9, growing in a mass surrounded by these unusual associates, still exists a small patch of oaks.

That we are here dealing with the recolonisation of deforested areas is perhaps hinted at by the intrusion of scattered low-lying stretches of the common bluebell (*Scilla nutans*), apparently quite at home with such strange associates as *Trientalis europaea*.

Thus we see that early in the nineteenth century we had in Lonsdale an alderwood continuous with the alders, willows and aspens marshalled along Lonsdale Beck, succeeded as we ascend the dale by a few oaks which were isolated by great stretches of larch, pine and alder from all contact with similar trees—and this is the position as it appears on the 1860 Ordnance Survey.

But the alders edging the stream, as we have demonstrated, like those in Ingleby Parish, Farndale and elsewhere, supported an *autumnata* colony which, as soon as circumstances admitted of the passage, colonised the new wood; their fate we shall consider below.

We must now return to Eston Moor; shortly after the foundation of the plantations in Kildale and Lonsdale the enclosure of common lands began, and amongst the land so enclosed were the portions of Eston Moor marked Wilton Wood and Normanby Intake Plantation on Fig. 10. Almost immediately irregular parts of the former were planted with larch and Scotch fir; these parts included a narrow wedge just south of the Beacon, narrow irregular patches skirting the lower boundaries of the enclosure, and a narrow strip with parallel sides joining up the former and otherwise disjointed parts. The upper part surrounding the Beacon was left untouched as was the broad central area, and both, when the 1860 Ordnance Survey map was produced, were heather and *Nardus* moorland and are labelled "Lighthouse Fields" and "Wilton Moor" respectively. Both supported (and still support) a few alders and odd birches around the Carr pools. Subsequently, some score or so of years later, the whole of the open portion of the enclosed area was planted with larch and fir.

Half a mile away, matters at Normanby and Upsall Intakes pursued

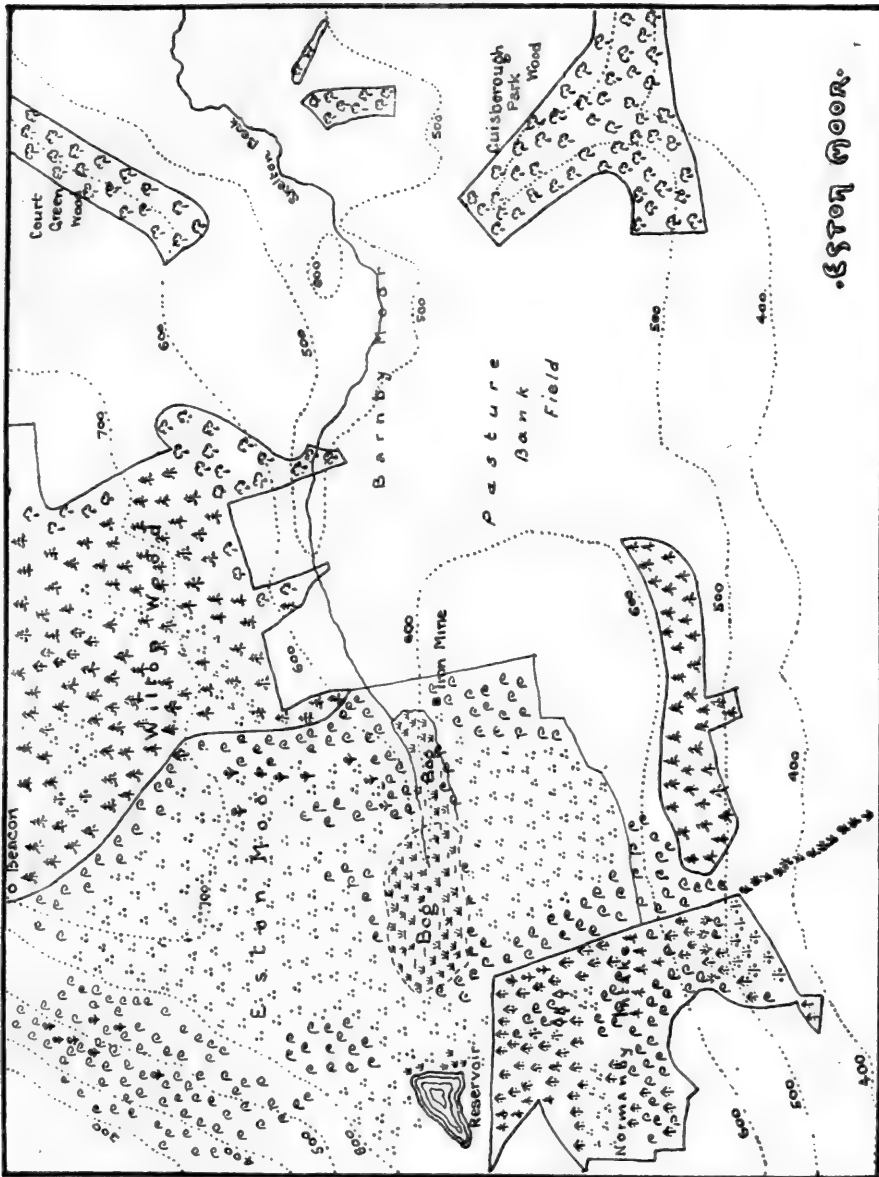


Fig. 10. Map of Eston Moor, Wilton pinewood and Normanby birchwood showing vegetation and contours.

a different course. Probably influenced by the fact that efforts had been made to bring the western end of the moor under cultivation in the middle ages renewed attempts were directed towards the same end. As advised in Marshall's *Rural Economy*¹, a row of birches was planted just within the northern boundary wall to act as a wind shield, and at the same time the land was broken into fields. Failure once more ensued as the soil composed of weathered Moor Grit is too light for successful agricultural operations, and the plan was abandoned. In order to avoid waste the "Intake" was then planted with larch and fir. Thus, in the early "eighteen hundreds," Wilton Wood and Normanby Intake Plantation were coniferous in character but included isolated clumps of birch and alder, and this was exactly the position when the 1860 Ordnance Survey was made. If any difference is perceptible between the two, it is that the latter wood is represented as being the more closely provided with coniferous trees.

By the very fact of the enclosures the alders along Skelton Beck and around the Moorland Carrs were left exposed and have been destroyed, only traces of their former presence being left. Thus early in the nineteenth century all communications between Wilton and Normanby Woods *via* the alders of the moor pools and streams were broken. Nevertheless, conditions within the woods still agreed; slope, elevation, exposure, vegetation quite coincided; the sole difference lay in the fact that Wilton Wood lay on the geologically younger sandstones of the Lower Estuarine series whereas Normanby Intake lay on the older Moor and Fossiliferous Grits.

Such agreement held until about the year 1885 when drastic changes took place in the Intake Plantation. A heavy north-east gale so damaged the trees that many broke off, and as the wood was ruined the remainder were felled. When I first visited the wood in 1906 the decayed stumps of those cut down still remained as did also the jagged trunks of those hurled down by the wind. Except at the extreme south-east (see Fig. 10) the only coniferous trees left were two seedling pines and one larch. In their stead were springing up in all directions crowds of birch saplings, obviously, from their north to south trend, proceeding from those originally acting as a wind shield; now (1919), almost the whole area once under pine and larch is occupied by birch. Fully one half of this ground has been gained since 1906. With this birch, around the old pools still survive the alders of times long gone by. In contrast

¹ Marshall, *Rural Economy of Yorkshire* (1796).

to this Wilton Wood, from a coniferous standpoint, is quite untouched, whilst birch and alder alike are vanishing.

From this we glean that the once continuous Eston colony of *Oporabia autumnata* was, about the year 1800, broken into two distinct portions separated by half a mile of heather and bracken; from that period until 1860 and onward until 1885 these two sections, although disjoined, continued to exist under identical environment. After 1885 the conditions fundamentally altered; one division had to live in a coniferous wood in which birch and alder were being suppressed, and the other in a birch-wood (with some alder) where conifers were disappearing.

With these changes corresponding changes of an evolutionary nature have occurred in the respective portions of the old *autumnata* colony which exhibit themselves especially in the following four features: (1) the mean sizes of the two races are different; (2) their food plants are distinct—birch in the one case and larch (rarely pine) in the other; (3) the Wilton Wood insect is a duller, feebly marked insect with its variation range swinging round this as a modal condition, whereas the birch insect presents us with diverse strains with an abundance of pale silvery and barred forms as well as of feebly marked forms including those just referred to; (4) the pinewood insect emerges in September and is quite over by the end of that month; on the contrary the birch insect rarely occurs in September, in which month I have seen but two wild specimens—a male in 1910 and a female in 1916; it only becomes abundant about October 10. So great was the divergence in this respect that for several years after my discovery of the insect amongst the larches, in spite of careful search conducted on the same days as I climbed the hill specially to secure material from the pinewood, I missed the birch colony and actually published a note to the effect that the insect did not occur there. Only a casual visit in mid October, 1909, in search of *Arachnida*, revealed its presence and demonstrated why it had escaped previous observation.

And the wonderful thing about these differences is that they must have arisen in the period between 1886 (when the environments ceased to be alike) and 1907, when my acquaintance with the insect commenced.

That they do not depend upon the ground vegetation seems obvious because amongst the larches, where alone the insect occurs in Wilton Wood, the plant associations differ in no important respect from those of the birch wood, as the following table, including for facility in reference the vegetation of the alderwood in Lonsdale, will show.

The ground vegetation thus not being the decisive factor, it seems

that for an explanation of the changes we must look to the passage to birch in the one case and to larch in the other.

Of this, in the case of size, we can adduce experimental proof very readily; a birch diet quickly restores the pinewood *autumnata* to its former greater mean size; nor need the question of changed food delay

TABLE III.

The vegetation of Wilton Pine Wood, Normanby Intake Birch Wood and Lonsdale Alder Wood.

Plant	Birch Wood (open)	Pine Wood (closed)	Alder Wood (has some open spaces)
<i>Pinus sylvestris</i>	R*	A	C
<i>Larix decidua</i>	R	A	C
<i>Betula alba</i> (agg.)	A	R	R
<i>Alnus rotundifolia</i>	O	R	A
<i>Salix caprea</i>	VR	VR	R
<i>Populus nigra</i>	—	—	R
<i>Quercus robur</i> (agg.)	VR	One	{ Common in one restricted area
<i>Pyrus Aucuparia</i>	R	—	C
<i>Crataegus oxyacantha</i>	R	VR	R
<i>Fagus sylvatica</i>	One	—	—
<i>Acer pseudoplatanus</i>	Odd examples	—	—
<i>Oxalis acetosella</i>	A	A	A
<i>Cardamine pratensis</i>	—	—	O
<i>Viola Riviniana</i>	C	—	—
<i>V. palustris</i>	—	—	C
<i>Stellaria holostea</i>	—	—	C
<i>Galium saxatile</i>	A	A	A
<i>Potentilla Tormentilla</i> (agg.)	A	R	C
<i>Calluna vulgaris</i>	C	LC	A (in open spaces)
<i>Erica Tetralix</i>	C	LC	C
<i>E. cinerea</i>	C	LC	C
<i>Vaccinium Myrtillus</i>	C	LA	LA
<i>V. vitis-idaea</i>	—	—	LC
<i>Empetrum nigrum</i>	C	C	—
<i>Trientalis europaea</i>	—	—	A
<i>Rumex acetosella</i>	A	A	C
<i>R. acetosa</i>	—	—	C
<i>Deschampsia flexuosa</i>	A	LA	LC
<i>Molinia caerulea</i>	C	—	—
<i>Agrostis</i> spp.	C	Not C	Not C
<i>Festuca ovina</i>	C	Not C	R
<i>Nardus stricta</i>	LA	R	VR
<i>Holcus mollis</i>	—	—	C
<i>Scilla non-scripta</i>	—	—	LA
<i>Luzula multiflora</i>	C	O	O
<i>Juncus</i> spp.	LC	L	LC
<i>Pteris aquilina</i>	LA	LC	A
<i>Blechnum Spicant</i>	R	R	R
<i>Lastrea</i> spp.	R	C	C
Bryophytes	A	VA	A
Fungi	A	A	C
Lichens	R	R	C

* The frequency symbols are:—A, abundant; C, common; O, occasional; R, rare; VR, very rare; LC, LA, locally common, locally abundant.

us. With the inhibition of birch and alder by the larches and pines the insect, if it had to survive, had to accept substitutes either in one or both of these. This it found in larch, upon which it subsists exclusively in the western part of the wood. Its birch instinct has not, however, been lost; about half a mile within the wood where a little island of birches lies surrounded by pine and larch, larvae can be beaten from either birch or larch. Neither of the first two differences is germinal, the other two stand upon a vastly different plane. Both are germinally fixed and both remain unaffected by any amount of rearing under a changed environmental complex.

In investigating the change in the modal state of the wing pattern one must guard against the assumption that any extension of the range of normal variation has occurred; what has happened, in reality, is a very definite contraction of that range of such intensity as to throw the modal condition into a position giving the two races facies so diverse as to suggest an apparent discontinuity. This, examination proves not to occur, for the alteration in mean proceeds directly from a preferential preservation of the darker, suffused, feebly marked forms and an accompanying elimination of the paler and the banded genetical strains existing in birchwood *O. autumnata* not only on Eston Moor but on Waldrige Fell¹, Co. Durham; in Cos. Tyrone and Fermanagh, Ireland; and Cos. Perth and Kincardine, Scotland.

To emphasise the fact that we do not here encounter any development of new varietal forms or range, just prior to writing the previous sentence I deliberately and without bias took all of my long series of pinewood insects and likewise those from the birchwood, and having examined them individually, assigned each to what I should have considered its point of origin had they been placed before me for judgment as being certainly from either pine or birchwood but exactly which unknown. So treated I should have allocated eleven birchwood individuals (10 males and 1 female) out of 71 (15.5 %) to the pinewood and six pine specimens (5 males and 1 female) out of 144 (= 4.16 %) to the birch. In connection with this test, in the case of these last examples a remarkable fact was apparent when I placed them alongside similar individuals derived from the birchwood. I found that they resembled the darker examples of their class, and further it was forced upon me that considered simply as Cleveland insects, with particular history unknown, they would have been deemed representatives of the alderwood in Lonsdale—a totally unexpected result of a casual test. Whence it will

¹ For the vegetation on Waldrige Fell, see Fig. 12, p. 239.

be seen that the pinewood race or races are derived from those in the birchwood by a process of selection working against the pale silvery insects which one looks upon as type *O. autumnata*.

Let us proceed to investigate the mechanism of this limitation. In the birchwood the silvery insects do most certainly blend best with the trunks upon which they rest; so, too, do the darker insects of the other wood with the dull-toned larch bark. But as a protection in the daytime this would be valueless should they be molested by tits or other insectivorous birds and like enemies. All of the insects, pale, medium or dark, at once betray themselves by flying off the trunks, even when the disturbing agent is yards away. Selection thus would not be preferential. Except for a solitary pair of wood pigeons, and once a thrush, the birchwood has to me always seemed void of bird life; matters, however, are different in the pines. There birds are much more prevalent, bullfinches and other birds abound, but during all my years of observation I have never seen a single *Oporabia* of any species attacked by birds by day! At night time the position changes; amongst the birches few if any nocturnal birds occur, for the trees are not well enough grown to harbour them. In the pines hosts of owls and night-jars aided by bats wreak terrific havoc on the insect life of the wood, as the number of lepidopterous wings and coleopterous elytra lying about show.

Recognising this fact, I have from time to time critically examined all of the detached *O. autumnata* forewings I have seen in the wood. In all I have noted 17; two of these, both dark, were entangled in the floccose web of the very abundant spider *Amaurobius fenestralis*, and 15 lay on the grass—in some cases in pairs. Those taken by the spider are negligible; chance ensnarement explains them. The other 15, in a population where the dark individuals outnumber the pale by more than 25 to 1, actually included a majority of pale wings! This cannot be a mere matter of chance; selection must be at work—and natural selection carried out by bats, owls and night-jars. These in the uncertain twilight of the wood at the flight-time of the moth would more readily secure the more conspicuous paler strains, thus progressively eliminating them. The observation that the paler examples yet existing were more heavily barred than the bulk of the parallel birch forms suggests that even the barred birch form itself is not an elementary strain, but was capable of movement in the mode when submitted to selection, either natural or artificial.

In my opinion, therefore, it is to natural selection, carried on by nocturnal birds in company with bats, we have to look to explain the

rapid change in average condition the insect has displayed in the pinewood.

But the change in the period of emergence is a matter much less susceptible of explanation. It cannot be mutational for three reasons: (1) it is universal in a wood of tremendous extent in which free communication between the preferred larch zones is barred by belts of pure pines, and, in one case, by a stretch of *Nardus stricta* moor; (2) had it been mutational no selective factor acting in favour of the new character can be at work, for it presents no apparent advantage aiding selection; (3) it affects both of the germinal strains occurring in the wood.

The second objection amongst these three also indicates that pure selection, whether by the agency of birds, bats, arachnids or ichneumons, acting on the race as a whole cannot explain the anomaly, even had I not put the matter to test by trying by selection to set up an early emerging strain from birchwood parents. Having secured most exceptionally a September birch female I bred from her earliest progeny in the succeeding year and continued the procedure for three generations, with the result that the last brood reared emerged on the average a fortnight *later* than birch broods from wild parents reared alongside them. The experiment was therefore discontinued. Even had it been a success artificially, and it is conceivable that it would have been had I continued for a hundred(!) years, in a state of nature, unlike what occurred in the evolution of *O. filigrammaria*, no advantage accrues from early emerging, so nothing presses the balance down in that direction. On the contrary a positive disadvantage manifests itself, for early laid ova of *O. autumnata* have been known to hatch the same year; larvae from these must perish when the trees shed their leaves.

We are thus forced to cast about for explanation in other directions. That we must look to causes operating within the pinewood, and therefore environmental, seems certain, for parts of the wood are as far apart as they are from the birchwood, and yet throughout the early emergence obtains.

Neglecting genuine pine and larch feeding lepidoptera, the most prominent insects in the wood are *Phigalia pedaria*, *Tephrosia bistortata*, *Gonodontis bidentata*, *Hybernia marginaria*, *Cidaria suffumata*, *Melanippe sociata*, *Larentia multistrigaria*, *Orthosia helvola* and *Xanthia circellaris*. Classifying these on the basis of food plants we find that the first four are not genuine larch feeders but have adopted it as food; three feed on *Galium saxatile*, and two are listed as feeding on trees not found in the wood and are presumably in the position of the first four,

but of this I have no proof. If we classify them according to their period of emergence seven appear between March and June (both inclusive) and always distinctly later than the same species at sea level or further south, whilst *O. helvola* and *X. circellaris* emerge in autumn, slightly in advance of their relatives elsewhere; their displacement, although in the same direction, is not so pronounced as that of *O. autumnata*. Nevertheless we have gained some sort of clue to the solution of our problem; the anomaly is connected with species hibernating as ova, aestivating as pupae and with autumnal emergence.

Increased temperature acting on the pupae may at once be ruled out; forcing in the genus acts as a retarding influence. But is the position the same if the ova hatch earlier than the normal? Is this early hatching accompanied by accelerated emergence? These points are readily put to the test. I took *autumnata* ova, forced them out in February and had them in pupae by March 28, exactly eleven weeks before their brethren in nature. And these pupae, kept under as natural conditions as possible, yielded their imagines simultaneously with their wild relatives! Still, precocious hatching ova may play their part as we shall discover later.

What then determines the development of *Oporabia* imagines if not increased temperatures? They emerge in response to exposure to a stimulus of a progressive fall in mean daily temperature, as I proved by submitting half of a brood of pupae to a gradual fall in low temperatures and the other half to a similar fall in higher ones. The former delivered their imagines first.

Here, apparently, we have got at the root of the matter, for owing to the dense masses of moss, pine needles and decaying *Lastraea* fronds retaining their moisture, and aided by the closer canopy overhead, the pinewood attains a lower maximum summer temperature and a higher winter one than the open, less moss-grown birchwood—and the difference is perceptible at once as one enters them. In consequence, the optimum period of exposure to low temperatures capable of stimulating pupal development is experienced sooner in the pinewood, and the insect emerges earlier. This dragging backward of the period of aestivation is bound to be aided by early hatching ova. Furthermore, such early ova would be more advantageously placed in the larchwood than amongst the birches, for the latter leafs later in the Cleveland as the following dates for two successive years will show: larch April 15, birch April 23; larch April 24, birch May 5. Thus any precocious individuals would die on the one and succeed on the other.

This early leafing in itself may be an active agent in causing ova to hatch early, for eggs laid on larch buds would be exposed to the elevated temperature due to the rapid metabolism of the expanding buds much sooner than those on birch and would therefore hatch earlier. In addition, the superior transpiration of *Larix* over *Betula* during winter and spring when both are denuded of leaves may, indirectly, serve to secure the same end.

This explanation would meet the facts of the case admirably, but the following fact must be emphasised; to grant its germinal fixation is to adopt the Lamarckian view. In my opinion this is the correct explanation, its fixation being brought about by its score or more years of incidence. Taken to a height of 1000 feet in Northumberland, at sea level in Yorkshire, at 300 feet in Durham, in Kent, London and Ireland, with wonderful uniformity the pinewood ova hatch the earlier, and their imagines develop the sooner—facts that render the evidence of my breeding experiments as to its germinal nature the more emphatic.

Whether it fits in with one's bias or not, with persistent unanimity every little point connected with the divergence in habit and instinct in these researches points to such features being genuine Lamarckian effects.

Before leaving the subject of the pinewood insect I must mention that in 1908 I beat several aberrational larvae from *Pinus sylvestris* which were ornamented with rusty patches approximating the larvae in appearance to the condition of such pine feeding larvae as *Ellöpia prosapiaria*; these have never occurred again, thus any study as to their genetic behaviour is rendered impossible.

The case of the Lonsdale alderwood race need not detain us long. There the conditions are not fundamentally different from the mean of those of the Eston birch and pine woods. We have the more open nature of the one and its less accumulations of moss and needles, coupled with the presence of owls and night-jars to act as selective agents, in conjunction with the abundant patches of larch and pine tending to preserve by selection the darker individuals, but much less rigorously than in Wilton Wood. Inevitably a progression of the modal condition towards darkness has been displayed, resulting in the production of a race including strains intermediate in many respects to the other two. But, nevertheless, the period of emergence is not significantly moved; the very rapid rise in temperature in the wood during June, July and August owing to its patchy, open nature, aided by the very late leafing (often late May) of the alder, definitely ensures this.

Only one further fact remains to be brought forward, and we are finished with the local races; in spite of the presence of many larches never once have I beaten a larva from that tree in the alderwood, yet every single individual of the few birches, whether in the open spaces or amongst the alders, has yielded the larva, which conclusively proves that the larch diet in the pinewood was only assumed as a last resort.

(d) *The case of Oporabia dilutata and its subspecies O. christyi.*

To describe in detail the pre-glacial migrations of these two forms here would be unprofitable; firstly those of *O. dilutata* agree too closely with those of *Apocheima hispidaria*, which I have worked out previously¹, and secondly the recognition of *christyi* as a subspecies has been too recent to admit of its present distribution being worked out, and thence its movements in the past deduced. We shall therefore confine ourselves to a brief study of the Cleveland local races of *O. dilutata*.

Laying aside the case of *O. christyi* as being impossible of study for reasons similar to those just adduced, its local habitats not being fully ascertained, we are left with two recognisable races. One of these is the ordinary suffused melanic form with a considerable range of fluctuating variation and the other a brilliant silvery form attached to the little island of oaks within the coniferous and alderwood in Lonsdale. Although separated by a ridge 1000 feet high, the nearest colony of *O. dilutata* to the latter is barely three-quarters of a mile away in Easby oakwood, where the species exhibits melanism of a most pronounced type.

As regards the oak island two views are possible: first that it represents the remains of Sir Charles Turner's unfortunate experiments in 1782; and second that, if the presence of *Scilla nutans* and *Holcus mollis* can be used as corroborative evidence, it represents the surviving portions of primaeval oakwoods, long since demolished otherwise by human interference, which have been subsequently isolated by the planting of alder, larch and fir.

In any case it must have been cut off for over a hundred years from the oaks in Easby and Airyholme Woods, with which of old it may have been continuous around the spur of the hill between Lonsdale and Kildale. Segregated from influences of the nature of directly induced and of infiltrated melanism so disturbing in these other colonies

¹ Harrison, "The Geographical Distribution of the Moths of the Geometrid Subfamily Bistoninae," *Naturalist*, pp. 317—320 (1917).

of *O. dilutata*, the insect here clings to the pale ground of the original exponents of the species which the others have abandoned.

We thus see brought into action on a minor scale that geographical isolation, which in parallel ways throughout the world and in a specially striking way in the volcanic valleys of the Sandwich Islands, has been so prolific in the production of new species.

(e) *The question of Melanism.*

We now turn our attention to the vexed question of melanism in the lepidoptera—a most striking genetical phenomenon and one that has progressed and is progressing under our very eyes. Of this phase of variation the genus *Oporabia* in all its species, but more especially in *O. dilutata*, affords excellent examples.

In our investigation, although primarily concerned with *Oporabia*, we shall not restrict ourselves solely to that genus but shall treat the matter from a broad standpoint, adducing instances from any genus or species capable of illustrating any given point; to do otherwise would be an ill-judged procedure, so productive are other genera in melanic species.

As to the inciting cause many theories have been devised; cold humidity, soil, light deficiency, food and smoke each having had its advocate in the past either in the crude form of direct cause or as indirect units in a more complex system. But the most plausible theory yet advanced was that urged by Tutt¹ in his paper on “Melandroism and Melanochroism,” and based in the interaction of moisture, smoke and natural selection. Despite the plausibility of this combination the operation of some of the other possible agencies is not to be dismissed lightly. Cold, for instance, so offhandedly cast to one side by Tutt, is the direct agent in producing the melanism in two cases to be referred to shortly. We may not agree with Walsingham that the necessity of blackish tones because they are absorptive of heat has, helped by natural selection, ended in their acquirement and fixation; too many instances of pale Arctic and Alpine lepidoptera can be cited to disprove its universal occurrence. Nevertheless, the “cold” theory is the only one explanatory of such cases as *Dicranura vinula* var. *phantoma* and *Poecilopsis isabellae*. Both of these insects are derived from forms with whitish ground colour, the former from the type *D. vinula* and the latter

¹ Tutt, “Melandroism and Melanochroism in British Lepidoptera,” *Entomologists' Record*, Vols. I. and II. (1890, 1891).

from *Poecilopsis pomonaria*. Both, likewise, when scale development is taking place are exposed to low temperatures, the former to the frosts of a Murmansk and North Finland spring, and the latter to autumnal frosts at an elevation of 800—1000 metres in the Alps of the Tyrol.

Now laboratory experiments on lines imitating these climatic conditions have been undertaken by Standfuss, Pictet, Federley, Merrifield, Fischer and others. Pupae just at the critical point in scale development were exposed to various low temperatures and as a result insects, sometimes, but not always, melanic, were produced. Most of these authors contented themselves merely with securing the aberrative forms, but Federley¹ tried to correlate the melanism with scale condition. He discovered that the temperature employed had not only lessened the actual number of scales, but, in addition, it had diminished the volume of the individual scales, and this is precisely the condition seen in *D. vinula* var. *phantoma* and *Poecilopsis isabellae*. Let us consider the effects of this; during histolysis and histogenesis in the pupae notable changes are taking place; tissues are breaking down and reforming themselves with a necessary elaboration of waste products which must be eliminated. One of the media of this removal is the vestiture of scales and hairs on the insect's body. Consequently, when the secretion of the haemolymph, which is the mechanism of the passage, flows or is injected into the newly formed scales, instead of supplying a large number of scales with a normal capacity it supplies few scales with small capacity. Therefore, if the secretion responds as usual to enzyme action, each scale present will receive a greater quantity of the pigment so generated than usual, which means that the insect would appear melanic. Melanism is not a case of the genesis of new colours but is quantitative. For example, it is quite well known that straw-coloured—nay almost white—*Melitaea athalia*, *M. parthenia* and *Brenthis euphrosyne*, as well as those almost black, can be captured; yet these almost diametrically opposed colours are precisely the same, only in the latter case the great quantity of the brown pigment produces an outward semblance to black.

This then serves to explain not only the natural forms of *D. vinula* var. *phantoma* and *P. isabellae* but also some of the artificial results of temperature experiments on *Lymantria dispar* and other species.

From this line of argument it would seem likely that, unless registered in the germ plasm, the melanism of the two natural instances would yield to simple experiment in the laboratory as does that in the

¹ Federley, "Lepidopterologische Temperatur. Experimente mit besonderer Berücksichtigung der Flügelschuppen," *Festschrift f. Palmen, Helsingfors* (1905).

artificial aberrations of *L. dispar*. If it does not, then we are dealing with a further Lamarckian effect.

Similarly one cannot deny that under artificial conditions moisture induces parallel phenomena, and by the same mechanism of scale reduction, but unfortunately such variation, unlike what occurs naturally, is equally liable to be accompanied by albinistic forms. Furthermore, the artificial state is pathological and not hereditary, being coexistent with damaged scales; in natural melanism such as the moisture-smoke theory seeks to explain the scales are as perfect as in the ordinary form. For the same and other reasons we must not delay to examine the other alleged stimuli.

Tutt's theory of the united effect of smoke and moisture stands on much firmer ground. Briefly, as summarised¹ by its author, it is: Humidity *produces*² melanism; the environment of the particular species determines how far and in what direction melanism may or may not be developed; *natural selection* may counteract, modify or *intensify* the tendency to melanism.

As a sort of corollary to this, to explain melanism in districts like the west of Ireland and Scotland far removed from smoky manufacturing towns, he suggested that there the necessary blackening of the resting places of the insects, which enabled natural selection to work, was obtained by the direct darkening of rock surfaces from exposure to heavy rainfall.

Later, however, Tutt appeared to shift his position; in place of the statement that moisture definitely produces melanism we find him stating³ instead that "Moisture plays an important if indirect part"—a vastly different position from the first one of direct cause and effect.

To include all of the cases of melanism occurring in the British Islands we may summarise Tutt's later views as follows:

- (1) By its indirect action on rocks, etc. moisture blackens their surfaces in districts remote from urban areas.
- (2) In urban areas such influence is augmented by the deposition of soot and its fixation by moisture.
- (3) Natural selection acting progressively in favour of melanochoirc forms resting in positions so darkened urges the species towards its culmination in total melanism.

¹ *Entomologists' Record*, Vol. I. p. 96.

² The italics are mine.

³ *British Lepidoptera*, Vol. I. p. 65, 1899.

And this, except for those who like Doncaster¹ have assumed a non-committal attitude, has come to be the opinion of every lepidopterist who has ventured to make a reasoned enquiry into the matter. For my part I can only say that I disagree completely, because I believe the theory to be erected on erroneous assumptions due to the acceptance of generalisation in place of particularisation as to our climatic conditions, added to a complete misconception of the actual condition of tree trunks and rock surfaces, both in the smoke zones and in other affected regions. In particular Tutt's crucial instance of smoke-blackened greenhouses is an extremely unfortunate choice. The blackening of white paints cannot be compared with the supposed darkening of tree trunks, for the former is strictly chemical and proceeds directly from the action between the sulphuretted hydrogen of coal smoke and the basic lead carbonate in the paint producing black lead sulphide according to the following equation:—



No one will venture to assert that light coloured tree barks contain lead salts!

Laying aside this particular statement as merely due to oversight or lack of exact chemical knowledge I adduce as the basis of my disagreement the appended facts which I shall discuss seriatim.

(1) Independent of such local melanism as that discovered by my friend Goodwin in Kent affecting *Boarmia consonaria* and *B. consortaria*, certain affected districts like that surrounding Middlesbrough (rainfall 24.9 inches), that near Newcastle (rainfall 28 inches) and the Moray Firth (rainfall 26 inches) are situated in the driest portions of our country. Alone this fact need not have excited wonder, but when one mentions that in each of the cases the type of melanism exhibited is peculiar to the neighbourhood the matter becomes of extreme importance.

For instance, near Newcastle and Middlesbrough *Boarmia repandata* and *Oporabia dilutata* are both melanic, yet no one could mistake say Middlesbrough *repandata* for examples from Birtley or Gateshead near Newcastle, so marked is the facies of the insect proper to each district. As one passes from the towns in both cases the melanism disappears and the insect becomes typical. In other words the melanism is strictly localised, and has arisen independently in its own special region irrespec-

¹ Doncaster, L., "A Collective Inquiry as to Progressive Melanism in Lepidoptera," *Entomologists' Record*, Vol. xviii. Nos. 7—10 (1906).

tive of what has happened elsewhere. Much the same holds true of Moray Firth *Triphaena comes*¹.

(2) Both in winter and summer I have made prolonged critical examinations concerning the colouration of the bark of the trees in Middlesbrough Park, in which vicinity more soot is produced and carried down to vegetation than in any other locality I have worked. I confidently assert that, except when jutting branches deliberately shut off the action of the rain, the trunks are not significantly different in tone from those in the rural districts of Durham and Yorkshire far removed from urban influence. The Scotch firs remained reddish grey, the white poplars greenish grey, the birches silvery, the ashes ashen and so on. The same is correct too of trees growing in the Team valley, North Durham, within the smoke zone not only of the local works of Birtley but of Newcastle and Gateshead, and in the Team valley melanism of a most aggressive type affecting hosts of species is rampant. To gain some kind of appreciation of the condition of tree trunks in the latter neighbourhood at night time, on March 9th, 1919, a starlight night with moon somewhat obscured, I walked down the valley from Birtley towards Gateshead and studied the trees. Their paleness was so much exaggerated that I found myself classifying pale sycamore trunks as silver birches until nearer approach revealed the specialised buds and twigs of the sycamore.

(3) In damp districts I deny that the effect of heavy rainfall is to blacken either rock faces or tree trunks. In my experience, gained in the west of Durham and Northumberland (rainfall 44 inches), in Ireland and in Scotland the moisture favours the development of algae, lichens and bryophytes which are far from being black in hue; in many cases the rocks become bright in colour owing to the presence of such highly coloured lichens as the very common bright orange *Physcia parietina*.

(4) Another fatal objection seems to lie in the fact that only insects resting in such blackened positions should be capable of assuming a melanic guise under the action of natural selection. This is not the case, and examples illustrative of the point are easy to obtain. For instance, *Larentia multistrigaria* (except very rarely) rests by day on the sheltered side of tufts of *Nardus stricta* amongst which its food-

¹ Tutt says that this has arisen by natural selection working on an insect resting on peat; my only objection is that it can't! Probably no worker save myself has ever had a dozen examples of the Moray Firth insect captured at rest by day; without exception these were taken from grass tufts and not from bare soil of any kind—peat or otherwise.

plant, *Galium saxatile*, creeps; in two localities, one in each of the areas I have studied, in Normanby Intake Plantation near Middlesbrough and on Birtley Fell near Newcastle, the melanic race *nubilata* is rapidly developing. The case is particularly impressive in the first named instance for, if by any chance the insect rested on bare ground—and I have never seen it do so although I have observed thousands of wild specimens—it would have to sit on a light coloured soil composed of weathered Moor Grit. And the same applies to the case (amongst others) of *Dasystoma salicella*, the males of which at Birtley exhibit progressive melanism in spite of their sitting in March on the light yellow grass culms of the previous year; this, too, holds with *Xylophasia monoglypha*, which almost uniformly hides itself at the base of *Nardus*, *Poa*, *Brachypodium* and similar plants, from which I have often dislodged it when in search of Arachnids.

(5) Furthermore, the same argument demands that melanic forms should not be generated when the insects concerned, whilst yet tree-frequenting, do not rest on dark surfaces; again the facts directly oppose the theory.

On Waldrige Fell, in the industrial district of North Durham, there is a colony of *Asphalia flavicornis* isolated far from any other colony of the same insect. This moth in its typical form has silvery grey wings and sits on birch trunks and twigs. Now the birches it haunts possess the usual silvery bark and purplish twigs, yet the insect there assumes an intensely melanic guise such as I personally have never seen elsewhere; nor have I seen it referred to in literature. On Eston Moor, where again the birches do not differ perceptibly from those seen by me in secluded Highland glens in Scotland, the insect likewise shows signs, not so emphatic as the fully developed melanism of Waldrige, but still quite unmistakable, of strong melanochoic tendencies.

To produce another instance is not difficult, although in this case the insect rests on alder leaves. Both in Lonsdale in Cleveland and in Chopwell Wood in Durham the moth *Melanthia bicolorata* is plentiful, and shows a great range of melanic and melanochoic aberrations, and in neither case can smoke blackening explain their dominance. The undersides of the alder leaves are no darker than those in Upper Allendale where the rainfall is 44 inches per annum, and the insects the whitest I know.

(6) Of the same nature but of different weight is the evidence yielded by woodland insects in the west of Scotland and Ireland. To

choose our own *Oporabia dilutata* as an example; it produces its silveriest forms in those very districts. Whatever melanism exists there does not affect woodland moths; it manifests itself in insects found on spray-drenched and open spaces, whether rocky or sandy in nature.

(7) Lastly, to demonstrate that the effect of natural selection is quite negligible as a factor in progressive melanism, I carefully studied the case of *Polia chi*, which in the Team Valley produces about 50 % each of typical and of the melanic forms grouped under the name *olivacea*, and near Middlesbrough about 10 % of dark and 90 % pale forms. For several years and on every day during their season—rain or fine—either my wife or my brother carefully noted the positions of the insects resting on three walls: (1) old and dark in parts, proceeding from Birtley to Newcastle; (2) old light yellow sandstone, proceeding to Burnmoor; (3) mixed new greyer sandstone and old reddish ones leading to Chester-le-Street. On these three walls I have seen up to three hundred examples daily, so that the present test is not confined to few insects; in the evening full particulars would be given to me, and sometimes alone and sometimes accompanied by my brother I would go over the ground to investigate the fate of the insects observed earlier in the day. Never was there any diminution of numbers in which more *olivacea* vanished than type *chi*; as a matter of fact we used to consider it a marvellous thing if even a single one had disappeared.

Now to proceed. The melanism both of the continuous type shown by *Larentia multistrigaria*, *O. dilutata* and *O. autumnata* as well as the discontinuous form encountered in *Amphidasys betularia* var. *double-dayaria* and *Boarmia consonaria* is germinally fixed, so that any possibility of its being ontogenetic is excluded; besides, except in the very unlikely case of its being possible to affect mature ova and spermatozoa by the same agency and in the same direction as the somata are affected, the impossibility of its being other than germinal is capable of absolute proof in many insects like *Spilosoma lubricipeda*, *Orgyia antiqua* and *Lycia hirtaria*. In all of these cases gametogenesis is completed long before scale and pigment formation, so that unless the germ plasm were in itself capable of giving rise to melanic forms the phenomenon would not be observed in succeeding generations. And if it did occur merely as a somatic aberration, then, not being germinal, selection either artificial or natural would not be capable of fixing melanism of the degree already attained, much less of urging it on to its climax in such totally melanic forms as *Oporabia dilutata* var. *melana* or *Gonodontis bidentata* var.

nigra. Owing to the evidence of *S. lubricipeda* and the other insects named above, we are thus forced to accept the position that the exact cause inducing the melanism acts or becomes potent for future action during the larval life of the insect, and with certain insects very early in that life. This incidentally explains why the so-called temperature aberrations cannot reproduce their kind.

The phenomenon being one initiated in larval life, it seems physically impossible for environmental moisture to act through the soma so as to affect at one and the same time the cells of the soma and those germinal in character. We are therefore driven to search for other causes.

I am quite aware that mechanical and physical disturbances, such as centrifugal force and X-rays, have been shown capable of producing somatic melanism, but each of these agencies acts at the so-called critical point of early pupal life, and by crippling special cells at the one time, and the whole organism at others. For these reasons it would be profitless to discuss them at length here.

Such being the case what have I to offer in their place? Let us consider what other drastic changes have been noted in the affected areas under consideration simultaneously with the appearance of melanism. The most striking is the decadence of cryptogams, more particularly of the mosses, liverworts and lichens, and to a less degree the ferns. This most certainly originates from smoke contamination. No one who has studied the cryptogamic botany of North Durham and North Yorks would fail to contrast the rich moss and lichen floras recorded for Long Acre Wood (1)¹ in the Team Valley by Winch ninety years ago with their paucity now. Of the lichens only the ubiquitous and apparently iron-constituted *Cladonia pyxidata* persists, and of the mosses only the accommodating *Fissidentes*, *Mnium hornum*, *Hypna* and a few others remain. The same holds good but to a less extent in Cleveland; there one would look in vain in the northern portions for the hosts of lichens listed by Mudd half a century ago.

But in the two cases differences are easily recognised; in the Durham locality, whilst matters with the mosses slightly ameliorate as we leave the soot zone, the apparent extermination of the lichens has been more complete. Besides *Cladonia pyxidata* I am only acquainted with two patches of well-grown lichens in the Team Valley, and both of these are *Parmelia saxatilis*. In the Cleveland as one leaves Middlesbrough the lichens reappear, *pari passu*, so that in the remoter dales the whole flora comprises the same species and in the same abundance as fifty years ago.

¹ The figures refer to those on the map shown on Fig. 11.

Nevertheless it must not be forgotten that a score of miles out the lichens are materially influenced. How has this been brought about? The difference in the two cases, coupled with the progressive reappearance as the towns are left behind, shows us that the deleterious agent at work is the smoke, and that the apparent exaggeration of its effects

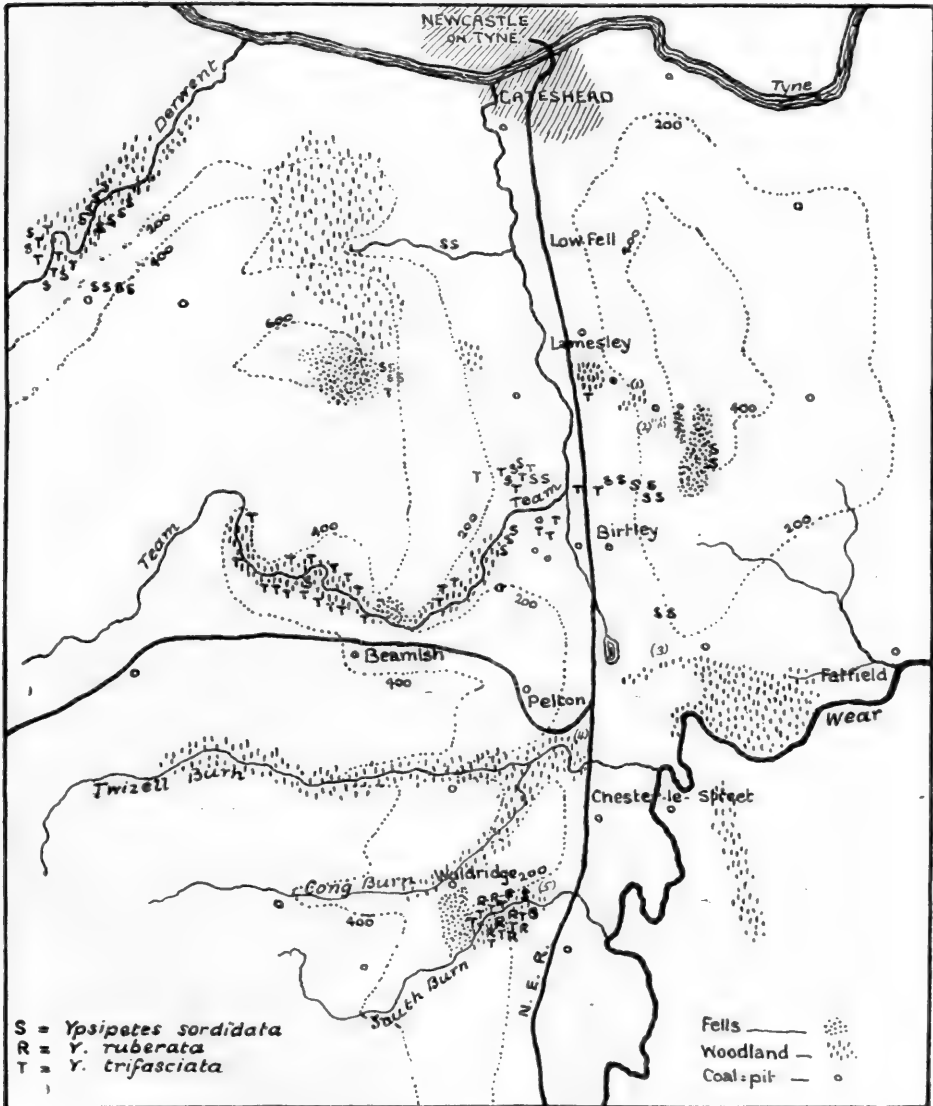


Fig. 11. General map of the Team Valley.

in Durham when compared with the conditions in Cleveland is due to the prolonged industrialisation of the more northern district and the comparatively recent development of Teeside Blast Furnaces and Chemical Works.

We must guard against assuming that the lichens are entirely gone, this is far from true, as careful search will indicate that many exist on walls and trees in the immature granulose condition. Their passage to the perfect condition has been prevented by the surrounding smoke and more particularly by the hydrocarbons of the ethylene series—not necessarily gaseous—it contains. Should anyone doubt the efficacy of this effect a simple test will demonstrate its truth. Plant a few sweet peas in a flower pot in the dark; let them grow until the etiolated epicotyl has attained some length. Then expose to town smoke. The epicotyl is at once flattened and very serious disturbances in growth ensue.

I have not elaborated this point simply to show that smoke affects vegetation but to show that such interference, whilst progressively diminishing as one leaves urban conditions, still possesses enormous powers at great distances from the source of the contamination. Now if the smoke, in addition to destroying vegetation, likewise conduces to melanism, then when continuous melanism develops it should likewise diminish as one leaves the town. Of the truth of this I have ample evidence drawn from both districts and seen in *Oporabia dilutata*, one of the very species we are studying. In Long Acre Dene Wood (1), probably the first due south of Gateshead and Newcastle to yield the insect in quantity, a good proportion are perfectly black, whilst those in Leyburn Hold Dene (2) are greenish black suffused forms, displaying the usual transverse bands; passing south of this to Lambton Woods (3) we find paler although still melanochroic forms; then finally we reach the Hermitage Wood (4) where, in addition to the Lambton forms, we capture ordinary pale typical insects.

In the same valley *Ypsipetes trifasciata*¹ shows the same progression in its melanism as we approach Gateshead from Walldridge Fell (5). In the latter locality the pale central band of the type still retains relics of its former silveriness, whilst just beyond Lamesley in the habitat nearest Gateshead all of the insects appertain to the completely black form known as *nigerrima*. The case of *Y. trifasciata* is worthy of special

¹ The "T" on the general maps of the Team Valley and of the Northern Cleveland shows the distribution of *Ypsipetes trifasciata*; the "R" and "S" give similar facts for *Y. ruberata* and *Y. sordidata*, respectively.

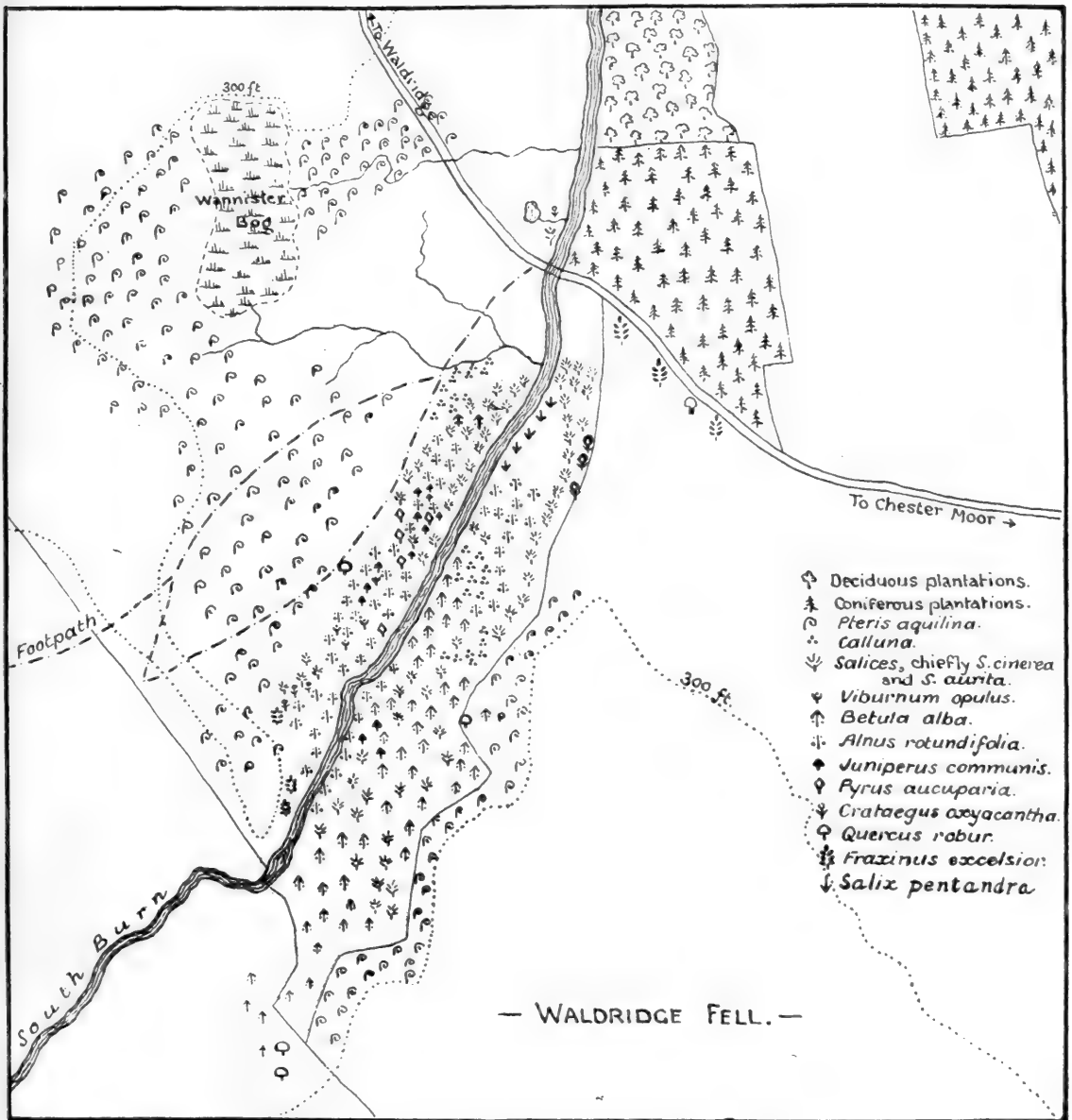


Fig. 12. Map of Waldrige Fell showing vegetation and altitudes.

attention because twenty-six years ago the insect was quite typical locally.

Near Middlesbrough *O. dilutata* is melanic right up to the base of the hills seven miles away; over the first ridge and not a mile away from the limit of melanism the moth is as pale as if taken in Ireland. In this district *Ypsipetes trifasciata* is more local; still there are two stations for it, one on the south face of Eston Moor and the other in Lonsdale, farther south. The insects from Eston are melanochoic whilst the others are quite ordinary.

But other species show the gradual decrease in melanism much more vividly as we recede from town conditions in Northern Cleveland. *Boarmia repandata* near the town is quite black; just three miles out it is grey, whilst specimens from Stokesley (see Fig. 13) are perfectly typical. So, too, near Middlesbrough and North Ormesby *Hybernia marginaria* exhibits total melanism; beyond a belt of three miles such a form is unknown.

Of the two areas the Middlesbrough one is the more striking; as we leave the borough the annual rainfall increases rapidly from 24.9 inches to 36 inches—reaching its maximum where the melanism is least. Instead of a direct relationship between rainfall and melanism an inverse one is manifested.

As a deduction from the facts laid down above I have satisfied myself on two points: (1) as far as urban areas are concerned moisture plays, if any, a very minor part; (2) the only agents correlated with the melanism in its incidence are the smoke and impurities thrown into the atmosphere through the springing up of great manufacturing towns. How the latter factor may conceivably act detailed consideration will determine; melanism in other than urban districts we shall reserve for treatment later.

Let us pause here to examine the chemical status of the black pigments found in the animal world generally, in order to equip ourselves for our proposed investigation as to their origin in the lepidoptera. These pigments known as melanins are not necessarily black; indeed, some are brown. They occur in skin, hair, wool, feathers, scales, muscles and various other parts of the bodies of animals of diverse affinities. In composition they are substances closely allied to proteins. In fact, as far as it is humanly possible to decide they are chemically the same as the humins, a series of bodies derived artificially from many albuminous substances by the action of enzymes or ferments.

Of the albumin-derived proteins tyrosin (parahydroxyphenylamino-

propionic acid) seems to be that utilised by enzymes to generate the pigment so universal in its occurrence in the animal kingdom. This, experimentally, not only by simple hydrolysis with acids, but also by the action of ferments like tyrosinase, develops coloured compounds of the humin type, and this is the kind of reaction we are to look for to account for pigment occurrence in lepidopterous scales and hairs. That means

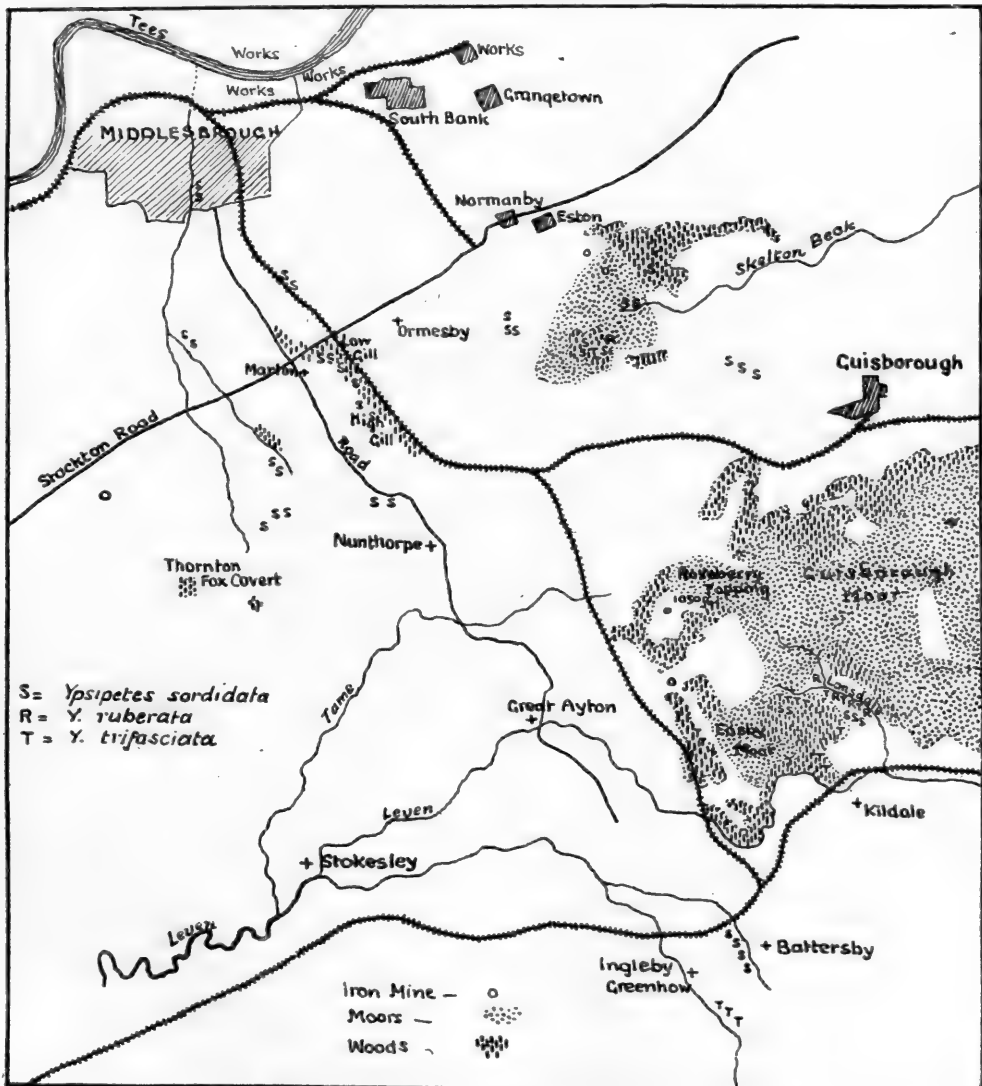


Fig. 13. General map of Middlesbrough District.

we have to demonstrate the existence in insects of such enzymes as tyrosinase and of protein-like bodies capable of yielding humins or melanins when hydrolysed, oxidised or submitted to enzyme action.

Proof of the latter point is easy; take a pupa of *Oporabia dilutata* and puncture the wing cases carefully. The light coloured haemolymph (yellowish in the males and greenish in the females) exudes; this on simple exposure to air shows the phenomenon of melanosis, that is to say it blackens. The haemolymph of insects is therefore endowed with compounds able under enzyme oxidation to yield black pigments.

It is equally easy to show that tyrosinase occurs in the haemolymph, but in this case, to secure adequate quantities for experiment, it is best to use large Sphingid or Saturniad pupae. Take say pupae of *Platysamia cecropia*. Pierce their wing cases; as before the pale haemolymph flows but naturally in much greater quantities. To this add ammonium sulphate and a precipitate is obtained. Dissolve this precipitate in a .05% solution of sodium hydroxide; now add this tyrosinase solution to one of tyrosin. Soon a violet colouration appears which gradually darkens and finally a black flocculent precipitate is thrown down, to be recognised as one of the humins.

This insect-derived tyrosinase reacts similarly to yield pigmentary compounds with many other benzene derivatives containing the hydroxyl radicle. In the haemolymph the tyrosin does not exist as such but in its place is a chromogen closely related chemically and conforming to the latter condition; it, likewise, when acted on by the enzyme tyrosinase yields coloured compounds.

Now to return to our melanism; it is perfectly clear that when we consider the great difference between a typical *Boarmia consonaria* and its melanic form var. *nigra* we are dealing with a profound derangement of the ordinary metabolic scheme; nor can it be denied that this disturbance is inherited, and not ontogenetic. These melanic forms, whether reared in the centre of affected areas or in some remote corner where such are never heard of, always breed true. Whence it follows that the original insect showing the new character, in this species, showed it by a sudden change in germinal constitution—or had its somatoplasm and germ plasm equally affected by some environmental impulse. The former possibility in its simplest form may be dismissed when due allowance is made for the fact that there is no reason whatever why melanism so produced should be localised; it should, on the contrary, be universal. But if the altered metabolism gives its possessor great advantages in dealing with unusual food the position would be greatly altered. And

the food is unusual in districts wrapped in a constant mantle of chemical smoke. Despite the truth of my observations that tree trunks are not significantly darkened every leaf (I speak from my knowledge of the Middlesbrough district) bears a film of smoke-derived impurities composed of organic compounds, both aliphatic and aromatic, and various salts of potassium, manganese, iron, sodium and other metals. If this altered metabolism is as I postulated fitted to deal with quantities of these ingested with the food, then without doubt it will endow its possessor with a considerable start in the race of life. That foliage thus contaminated is injurious to lepidopterous larvae may be readily demonstrated. If larvae of such a species as *Asphalia flavicornis*, originating in some remote Scotch wood, are reared on birch grown in Middlesbrough they die one by one until all are gone. But it is quite probable that such a radically changed metabolism as that laid down in our theory above would render insects endowed with it more capable of dealing with smoke-borne impurities; so that those not possessing it would commence life under a severe handicap when submitted to urban conditions, and thus their ultimate supplanting by their affected relatives secured. Under this hypothesis the smoke itself would not directly induce the melanism but would in the end secure its being supreme.

There remains still another possibility; just as in Firth's¹ and MacDougal's² experiments the injection of various salts into the ovaries of such plants as *Epilobium roseum* and *Oenothera odorata* is stated to have produced noteworthy aberrational forms, so the smoke-derived chemical compounds, organic and inorganic alike, may have affected the germ cells of the insect. This action, whilst presumably most potent in early larval life when relations between somatic and germ cells are most intimate, could work at any larval stage; by its action the chromosomes, whether by detachment, replacement or removal of the side chains of their essential nitrogenous compounds, could be so altered in powers as to cause the setting up of a type of metabolism quite foreign to the insect; this could be the type just discussed. Such a course need not be that pursued by all of the insects under town influence any more than all of Firth's and MacDougal's plants bore seeds yielding mutations; such insects as did follow it, whether selection worked on colour, on constitutional hardiness, or on better equipment for dealing with foreign

¹ Firth, "An elementary Inquiry as to the Origin of Species," *Journal Army Med Corps*, Vol. xvi. pp. 497—504 (1912).

² MacDougal, "Alterations in Heredity induced by Ovarial Treatments," *Bot. Gazette*, Vol. LI. pp. 241—257 (1911).

elements in the normal food, would in time be destined to replace the type since we have demanded that the germ cells are altered in potentiality and since we know that the germ plasm is continuous.

Now have we any proof of the possible existence of such a scheme of changed metabolism and of its not being disadvantageous to the organism involved? Not in the Insecta save by inference; we have its occurrence definitely¹ in the case of human beings.

In man occurs such a disturbance known as alcaptonuria. In this condition the urine becomes very abnormal; such bodies as tyrosin and its allies instead of having their benzene nuclei completely disintegrated by the slow combustion of vital processes, break down and yield intermediate products like uroleucic and homogentisic acids which when laid aside and submitted to hydrolysis or oxidation yield dark coloured pigments. These bodies, respectively dihydroxyphenyl lactic acid and dihydroxyphenyl acetic acid, are benzene derivatives possessing the necessary hydroxyl groups qualifying them for being acted upon by tyrosinase.

A person with metabolism of this curious type cannot be regarded as diseased; he lives and enjoys life quite unhandicapped. Any natural selection would fail to act against him; nay, should ever food be presented to him better dealt with by his than by the normal method then he would be the one with a start in life's race. A very important feature about this affection is that the available evidence² directly states that the condition is inherited.

There is thus a very suggestive parallelism between this disturbance and that formulated as the possible cause of melanism.

Grant then that, in the haemolymph and in the excretory products it contains after the reconstruction of the pupae succeeding histolysis, we have greater supplies than the normal of compounds for the insect tyrosinase to work on, then another fact favours the deposition of a melanin in quantity. Experiment has shown that ferments such as maltase have their action greatly accelerated by the presence of small quantities of such salts as potassium and sodium chlorides, manganese sulphate and so on. Have we not such present? They, especially the alkaline salts, must be taken in quantity on town grown foliage and cannot be excreted normally. They can thus during pigment formation affect the quantity produced and thus darken the insect.

We shall now return to such cases as the melanism of *Boarmia*

¹ And locally in the melanosis of horses, particularly of grey ones.

² Kirk, *Brit. Med. Journ.* 2, 1017 (1886); *Journ. Anat. and Phys.* 23, 69 (1889).

consonaria and *B. consortaria* in Kent. It is quite possible that there London smoke deposits its last impurities, but not likely in spite of the fact that the Lancashire smoke affects Lake District lichens seventy miles away. So small is the area affected that I think we are dealing with the effect of some trivial, local cause¹ of an obscure nature leading to the necessary metabolic upset. Here perhaps it is best to remark that the very great similarity of the melanic forms of the closely related species *Tephrosia bistortata*, *T. crepuscularia*, *Boarmia consonaria*, *B. consortaria*, *B. repandata* and *B. gemmaria* suggests that we are dealing with an atavistic character, and that the chemical change in the nucleus necessary for the reappearance of the old common form may not be a very profound one—a view that receives some little confirmation from the fact that the *cognataria* form of *Amphidasys betularia* found in all three of the known relict stations of Miocene forms, China, Eastern North America and Turkestan, shows much more pigment than type *betularia* and thus shows some approach to the melanic *double dayaria*.

Lastly we have to deal with the melanism and melanochoism of such species as *Campptogramma bilineata*, *Dianthoecia conspersa*, *Triphaena comes*², *Melanippe fluctuata*, *Enmelesia albulata* and others in habitats along the west and north-west coasts and islands of Ireland and Scotland in particular, and to a less marked extent along coasts elsewhere. All the species feed on low-growing plants, and all show their melanism chiefly in coastal habitats; none are woodland species. Not even our very impressionable *O. dilutata* is included; had the melanism been of the usual type it was bound to join in, yet in these very areas wherever woodlands approach the sea the perverse insect, as if to defy the moisture theory, will persist in giving us the silveriest of silvery forms.

What have they in common? All live on spray-washed food and all therefore receive great quantities of the salts found in sea water of the type of sodium and magnesium chlorides, bromides, iodides, sulphates and similar salts so that conditions approaching those in urban areas are set up, possibly with the same effects. This, too, may be the cause of the melanochoism exhibited by other coast Noctuidae, its possible accentuation in the more insular Scotch and Irish stations being brought about by their more exposed and isolated positions giving greater scope for its advent and its subsequent predominance by inbreeding.

¹ It cannot be chance, for two species are affected.

² I know that *T. comes* is melanic at certain inland stations, but these forms only exist where infiltration from the coast is possible; the metropolis, so to speak, of the melanism of that species lies on the Scotch coast between Burghhead and the Orkney Islands.

III. BREEDING EXPERIMENTS.

(a) *Inheritance of Subspecific Characters and of those characterising the Local Races.*

In any enquiry directed towards elucidating the evolution of subspecies and local races nothing is more important than to determine whether such differences as they present are ontogenetic in character, i.e. developed anew in each generation in response to their own peculiar environmental influences or not.

The first experiment I initiated along these lines was conducted with the subspecies *Oporabia filigrammaria*. I obtained ova of this form from wild parents captured at an elevation of 1500 feet on one of the Lancashire moors not far from Burnley. These ova were wintered almost at sea level at Middlesbrough, North Yorkshire. As they hatched the resulting larvae were placed on birch, alder and other foods proper to *O. autumnata*. Without exception they accepted these, to them, unusual foods and fed up speedily and healthily, the pupae emerging toward the end of August and the beginning of September just when their relatives would be coming out amongst their native heather. Not a single sign of departure from their own racial characters in the direction of those of *O. autumnata* was manifested either in larval colouration, imaginal size, wing markings or in other features. The race was inbred and kept going under similar conditions for five successive seasons and the insects bred each year carefully scrutinised for any possible development of *autumnata* characters; never at any time was any hint of deviation from their own normal state given. The experiment was repeated, only in this case the larvae from wild moorland females were reared at an elevation of three hundred feet in North Durham, in a district not only producing *autumnata* but also a race of *filigrammaria* of characteristic type; again no *autumnata* characters appeared nor were there signs of passage to the local *filigrammaria* form. Obviously from these experiments only one conclusion can be drawn and that is that the subspecific and racial characters in *O. filigrammaria* are germinally fixed.

Here it is well to remark that never at any point in the half dozen years of inbreeding that this experiment involved were any traces of diminution in vigour displayed; inbreeding with this species¹, unlike

¹ And the same held true of birchwood *O. autumnata* when inbred for a similar period.

what is experienced in *Zonosoma orbicularia*, seems most emphatically not to injure the race.

The reciprocal experiment of attempting to feed larvae obtained from birch-fed *autumnata* females on heather was carried out in the North Durham area with negative results as far as it went. The larvae attained the fifth instar and in that, as well as in the preceding four, they could not be distinguished from *autumnata* treated in the usual fashion. Owing probably to the difficulty of keeping *Calluna* fresh, and the consequent necessity of supplying them occasionally with stale food, they dwindled and died; not a single pupa was obtained.

Similar transplantation experiments were made in the case of the feebly marked pinewood race of *O. autumnata*. Ova from a mixed selection of wild females from Wilton Wood were brought down to Middlesbrough, where instead of 800 feet, the elevation above sea level is only 30 feet. When they hatched the young caterpillars were offered birch instead of their own larch or pine; without any hesitation they took it and were successfully reared. As a result imagines were obtained which in every characteristic marking of the race were indistinguishable from those captured in the pinewood; and this resemblance extended to the period of emergence of the imago which, against all probability, happened towards the end of September, just as did that of its wild relatives. But one difference was recognised; the imagines averaged a millimetre and a half larger than the wild specimens and now possess a mean size equal to that of the birch-frequenting race. The experiment was continued for three more years but no further change was perceptible. Except in size—a character in which they always agreed with the birchwood broods reared alongside of them—the evidence they have to offer is precisely that of *O. filigrammaria*, i.e. that the racial characters are germinally fixed; the size is ontogenetic in this instance.

Portions of four broods taken wild in the same pinewood were removed to the Team Valley, North Durham, where they were submitted to a hawthorn diet and were kept going for two years; the results were of the same order as those obtained in the last experiment. An increase of size but a retention of other characters, including those of habit, was exhibited. Clearly the increase in size depends on a removal from the less nutritious pine and larch to the more favourable birch or hawthorn.

In a further experiment ova from the Lonsdale alderwood (elevation 850 feet) were allowed to winter naturally in Middlesbrough and then reared (for three generations) under the same conditions as those used in the case of the pinewood race; again the racial characters were

absolutely immovable. In this instance I mixed the last domesticated brood with a random lot from the birchwood, and having shown him a wild and similarly chance lot of alderwood insects I submitted the mixture to a competent lepidopterist for separation. This he did most efficiently, not a single error being made despite the great natural convergence in facies of some individuals in the two races.

It is worthy of note now, that all friends to whom I have sent ova from various *autumnata* stations known to me always give it as their weighed opinion that any of the darker races prove the easier to rear; the birch feeding insect proving especially tricky in its behaviour. This coincides exactly with my own experience with the same insects and with my difficulties with larvae from pale Irish and Scotch birch females. In fact, although it is morally certain that I have bred more of these insects than any other worker, out of batches of ova from at least two dozen Irish females I have never reared a single imago.

To turn now to experiments with *O. dilutata*; ova from the very white strain attached to the Lonsdale oak island, from Easby Wood melanic females, from pale Irish females and from strigated females taken to the west of Middlesbrough were brought into Middlesbrough. The ova hatched in due course, and the larvae were reared there on food obtained just outside the borough boundary. This was continued for three seasons. In each and every case the insects bred true to their own type and showed the same range of fluctuating variation as it did.

Finally I obtained ova of *O. christyi*, the smaller subspecies of *O. dilutata*, from Ireland and reared them at Middlesbrough with *dilutata* and our own form of *christyi*, which is grey and less silvery in tone. Two years' rearing under the same conditions induced not the faintest trace of convergence; nor was one expected, for Nature conducts parallel experiments in the two localities known to me which produce both *dilutata* and *christyi*, one near Seamer in Cleveland and the other in Durham; in the former *christyi* is light grey and *dilutata* melanic, in the latter *christyi* is of the very pale type and *dilutata* of the medium grey tone, and they have always continued so as long as I have known them.

These observations demonstrate conclusively (1) that the differences between *christyi* and *dilutata* are germinal; (2) that their physiological reactions toward the agent inducing melanism, whatever it may be, are quite different.

(b) *Inheritance within the Limits of the Local Races.*

When I first conceived the notion that the *autumnata* races attached to the Wilton pinewood and the Lonsdale alderwood had been derived from that found in open birchwoods or alders in Cleveland and elsewhere, it became of paramount importance to determine whether the main birch types linked up by the apparently continuous fluctuating variation were inherited or not; whether any given female either gave or tended to give the whole range of variation; or, to state the problem once more, whether there were genetically diverse strains existing within the birchwood population. To answer my own question I captured three wild females, one a beautiful, clearly lined specimen with a pure white central area, a second of the strikingly barred *sandbergi* form and the third a dull expressionless grey with obliterated markings, and reared their progeny. In all cases the offspring leant most unmistakably to their mother although some little approach toward other forms existed—a transition slightly more noticeable in the brood from the barred female. From pairs selected to be as nearly like their parents as possible further broods were obtained with a repetition of the previous year's results save that fluctuating variation seemed more steadied. After a further generation producing evidence of the same value the experiments were discontinued save in one instance. Reciprocal crosses between the insect with the white ground and melanochoic forms of the species *O. dilutata* were made; in both crosses the hereditary nature of the parental condition was perfectly obvious, the peculiar *autumnata* character proving almost dominant. Further evidence to the same effect was obtained in the experiment in sex-linked inheritance described below, where the male employed was of the weaker *sandbergi* form; that its characteristics are germinally impressed the condition of the F_2 generation emphatically proves.

Thus we have abundant proof that the population of the birchwood is mixed, being composed of strains genetically diverse and therefore capable of isolation when submitted either to artificial or to natural selection.

(c) *The Inheritance of Melanism.*

My experiments in this direction were very uninspiring as regards their indications. I paired a melanic Middlesbrough male with a white Irish female and reared the result of the cross to the F_2 generation.

My experience repeated that of Prout and Bacot¹ in their *Acidalia virgularia* work; to the very end a blend was obtained. So emphatic was this persistency in blending and the absence of any hint of segregation that the only explanation possible is that based on permanent gametic blending. This therefore agrees perfectly with what one sees whenever the inheritance of melanism of a perfectly continuous type is investigated, and contrasts violently with the uniform evidence of segregation seen in cases of discontinuous melanism like that of *Boarmia consonaria*.

(d) *Sex-linked Inheritance.*

Although I quite recognise the assumptions underlying the use of the word "sex-linked" as applied to the particular type of inheritance I am about to consider, in my opinion the use of Morgan's² word is preferable to that of "sex-limited" which covers, as Darwin intended, phenomena quite different from these.

That sex-linked inheritance of certain elements in wing-banding was exhibited by the moth genus *Oporabia* was first indicated in 1914 when I reared reciprocal crosses between *Oporabia dilutata* and *O. autumnata*; it was then perceived that whereas the males partook of the wing-markings of both species, the females, on the contrary, resembled the female of the species supplying the male participating in the original cross. In the case of the hybrid between *dilutata* female and *autumnata* male this resemblance was perfect but unfortunately only one F_1 female was bred and the matter was not proceeded with. Still worse was the predicament in the reverse cross where the influence of the male was not so perfect; here the F_1 females were devoid of ovaries. Furthermore, the back crosses involving both F_1 males proved almost completely unproductive. Very powerful barriers were therefore set to investigations employing these interspecific crosses. Knowing that such anomalies in sex condition did not exist in crosses between *O. autumnata* and *O. filigrammaria* exhaustive experiments were undertaken with these two forms, but they proved utterly fruitless.

Light, however, was thrown upon the subject in a very unlooked for fashion by breeding results involving the Carpet-banded mutation of *O. autumnata* which I shall call *latifasciata*. In this mutation the whole of the central area between and including the two median bands

¹ Prout and Bacot, "On the Crossbreeding of Two Races of the Moth *Acidalia virgularia*," *Proc. Roy. Soc. London*, Vol. LXXXI. Series B (1909).

² Morgan, "Sex-limited and Sex-linked Inheritance," *Amer. Nat.* Vol. XLVIII. (1914).

becomes blackish in extreme cases and suffused in those less so. Naturally the acquisition of a wild female¹ of this form, the first I had ever taken in the birchwood, caused me to speculate as to its genetic potentialities, so I paired it with a medium example of the barred form reared indoors. Anticipating with some confidence its dominant nature, I looked forward to an F_1 brood entirely of *latifasciata* form and including the first males of the type ever known. My expectations were utterly falsified for in 1917 in a brood comprising 47 individuals I obtained 22 perfectly typical females without the faintest sign of a complete bar and 25 males, all of which displayed the bar in the diluted form, showing incomplete dominance. This, *prima facie*, was evidence of that type of sex-linked inheritance known as "criss-cross." Consequently, in addition to pairing the F_1 insects *inter se*, all of the crosses including this F_1 brood, the insect in its typical guise, and the allied species *O. dilutata* were made. All of the intraspecific crosses yielded fertile ova as also did two of the matings of the F_1 ♂ and *O. dilutata* ♀; on the contrary, after a disheartening waste of material, none of the ova laid in the cages containing F_1 ♀♀ and *dilutata* ♂♂ changed colour, and were therefore infertile.

My 1918 cultures therefore were the results of the following pairings:

- (1) F_1 ♀ × F_1 ♂.
- (2) F_1 ♀ × *O. autumnata* ♂.
- (3) *O. autumnata* ♀ × F_1 ♂.
- (4) *O. dilutata* ♀ × F_1 ♂.

The first two of these were exceptionally successful and yielded important results harmonising exactly with what was anticipated should the inheritance be of the sex-linked type; nor was there any discrepancy between the results of the last two and one's expectations, only the number of imagines bred in these two cases was very meagre—just

TABLE IV.

Brood	Number of <i>latifasciata</i> females	Number of type females	Number of <i>latifasciata</i> males	Number of type males
F_1 generation ex <i>latifasciata</i> ♀ × type ♂	—	22	25	—
F_2 generation. (Same cross) ...	18	21	19	18
F_1 ♀ × type ♂ ...	—	43	—	36
Type ♀ × F_1 ♂ ...	2	1	1	3
<i>Dilutata</i> ♀ × F_1 ♂ ...	—	—	2	—

¹ I have taken three *latifasciata* forms in Yorkshire and have heard of one taken in Fermanagh, Ireland, and all were females; the similarity of this with the case of *Abraxas grossulariata* studied so exhaustively by Doncaster seems noteworthy.

what was expected from the last but certainly not in the third case. The exact composition of the broods, with that of the F_1 generation to complete the series, is appended in tabular form.

To anyone acquainted with the work of Morgan¹, Doncaster² and others and the ratios obtained in their sex-linkage work, this table adequately demonstrates that here we are dealing with a case where the male is sexually a homozygote and the female a heterozygote, and that the gene for *latifasciata*-ness is coupled with, or follows the distribution of, the male sex gene, or is located in the X chromosome, to give all of the various expressions used by different workers according to the views they accept.

In the conventional fashion, by the use of symbols, we can render this clearer by allowing L to stand for the *latifasciata* factor and A for its absence, or rather for the ordinary *autumnata*-ness. Then, since the original wild female was not pure for the character concerned, for its ova gave rise to both *autumnata* and *latifasciata*, we shall have to indicate its genotypic composition as $AL\text{♂} \text{♀}$ producing the two kinds of gamete $A\text{♀}$ and $L\text{♂}$ in equal numbers, and that of *autumnata* male as $AA\text{♂♂}$ yielding gametes all $A\text{♂}$. This would ensure that on the fusion of the gametes producing the F_1 generation equal numbers of two zygotic types $AL\text{♂♂}$ and $AA\text{♂♀}$ would appear. The latter appeared as typical females and the former as the heterozygous *latifasciata* males. At gametogenesis the $F_1\text{♂♂}$ produce two kinds of gamete, $A\text{♂}$ and $L\text{♂}$, and the females likewise two, $A\text{♂}$ and $A\text{♀}$. Now how does this constitution of the F_1 gametes square with the results in the F_2 generation and in crosses 2, 3 and 4? In cross 2 we mate a pure *autumnata* male, therefore yielding gametes all of composition $A\text{♂}$ with an $F_1\text{♀}$; these meet in fertilisation on our hypothesis with gametes constituted $A\text{♂}$ and $A\text{♀}$ and thus develop zygotes of the forms $AA\text{♂♂}$ and $AA\text{♂♀}$ which are ordinary *autumnata*—and precisely what the experiment yielded. Again in cross 3 a heterozygous *latifasciata* male was paired with an *autumnata* female. In this instance the male gives gametes of the two types $A\text{♂}$ and $L\text{♂}$ as does the *autumnata* female, but in this case they are of composition $A\text{♂}$ and $A\text{♀}$; all four gametes are generated in equal quantities. Theoretically from this mating zygotes of four kinds, $AA\text{♂♂}$ (*autumnata* males), $AL\text{♂♂}$ (heterozygous *latifasciata* males), $AA\text{♂♀}$ (*autumnata* females) and $AL\text{♂♀}$ (heterozygous *latifasciata* females) should appear in equal proportions. As we

¹ Morgan. *Heredity and Sex*. Columbia Univ. Press, New York (1913).

² Doncaster. *The Determination of Sex*. Univ. Press, Cambridge (1914).

see, four types are obtained; but whilst quite in accord with the prediction of equality when their paucity is considered this approximation cannot be deemed, in view of the small numbers, as very weighty evidence in favour of that equality.

Genetical analysis of the interspecific cross between *dilutata* female and F_1 *latifasciata* male need not delay us because the insects are too few to base any argument on.

There only remains then the F_2 generation to discuss; to bring it into being an F_1 male, producing gametes $L\♂$ and $A\♂$, was mated with a female of similar origin postulated in our scheme to yield equal numbers of gametes $A\♂$ and $A\♀$. This, as one readily perceives, agrees exactly with the conditions in cross 3; naturally therefore the breeding results should be similar, i.e. four zygotic forms, heterozygous *latifasciata* males, *autumnata* males, heterozygous *latifasciata* females and *autumnata* females, should be obtained in equal numbers, as was indeed the case, the exact figures being 19 *latifasciata* males, 18 *autumnata* males, 18 *latifasciata* females, and 21 *autumnata* females. And the numbers dealt with are great enough to exclude any action due to mere coincidence.

It will be noted that two possible pairings are not included; these are between heterozygous and homozygous *latifasciata* males on the one side and *latifasciata* females on the other; the reason is obvious; until *latifasciata* males had been secured simultaneously with similar females neither pairing was possible, the first becoming so in 1918 and the latter in 1919.

The complete agreement between predicted and actual results of the experiment thus serves to confirm the view that the gene determining progeny of the *latifasciata* type shares the distribution of the male sex factor, and furthermore that the original female was heterozygous for both, thereby confirming Doncaster's *grossulariata*—*lacticolor* results. Incidentally the fact emerges that a female homozygous for *latifasciata*-ness can never exist unless some further mutation determining a change in location of the gene for that character takes place.

We shall now take up the same question in connection with interspecific crosses between *O. dilutata* and *O. autumnata*. As was indicated above, reciprocal crosses between *autumnata* and *dilutata* exhibited inheritance on a sex-linked basis, apparently involving every gene determining wing markings in the cross between *autumnata* males and *dilutata* females, and only some in the reverse cross. But, unfortunately for the genetical analysis of these cases, in the latter all of the females

emerge months before their brothers, whilst in the former only one female was bred and was killed for dissection before its unique nature had been observed. Nor was the position of the males much better; their fertility was not greatly inferior to that of the pure species, but, on the contrary, the viability of any zygotes in the production of which their gametes took part was so slight that of the four possible back crosses only two gave larvae proceeding beyond the first instar. Moreover, of these two sets, members of only one managed to pupate, and it gave only five male pupae which died shortly after their maturation divisions commenced. Progress with these back crosses seemed impossible; nevertheless, in the hope that success might crown further efforts, they were repeated in 1915, when the families repeated the conditions of the previous season in every respect, one isolated female, wholly of *autumnata* facies, appearing in the *autumnata* ♂ × *dilutata* ♀ brood. This was paired immediately with an ordinary *autumnata* male and in due course 120 fertile ova agreeing exactly in size, colouration and sculpturing with genuine *autumnata* ova were deposited. They hatched in 1916 and gave rise to a brood of 87 imagines (50 males and 37 females); these proved in all respects and at all stages indistinguishable from pure *autumnata*. Crossed back with *autumnata* of pure wild blood and paired *inter se* they produced the same insect in 1917. This type of behaviour is susceptible of analysis and explanation as employed in the symbolical consideration of cross 2 above. It is probably a genuine case of sex-linked inheritance; still, a warning in this case is obligatory. The case is complicated by the absolute failure of reduction division in the gametogenesis of F_1 *autumnata* × *dilutata* hybrids, reinforced by the fact itself that only one female appeared in each of two broods.

Anomalies in chromosome behaviour other than the typical mechanism of sex-linkage may be at work. Furthermore, in 1917, when once more a single female was reared from several broods of the same hybrid, the female was half-sized and on pairing yielded no ova; subsequent dissection proved it to lack any trace of ovaries.

(e) Hybridisation Experiments.

(1) Hybrids between *O. autumnata* and *O. filigrammaria*.

The F_1 generation.

As we have insisted at several points above, *filigrammaria* begins to emerge from the pupae toward the end of August and continues to do so in diminishing numbers until mid September. Add to this the

noteworthy fact that forcing operations in the genus *Oporabia* are futile, and the possibility of crossing the two forms becomes very remote, but still not absolutely excluded. Precocious individuals of *O. autumnata* and belated representatives of *O. filigrammaria* occasionally overlap and therefore, if of opposite sexes, can be mated. Now this acts rather curiously in the matter of reciprocal crosses. In all of the species in the genus the males precede the females, with the necessary result that such overlapping specimens usually include female *filigrammaria* and male *autumnata*, and that combination is the one usually secured. However, in excessively rare instances, one can cage up *filigrammaria* males and phenomenally early *autumnata* females as I did, for the second time in my long continued work on the genus, in September 1918.

When the opposite sexes of the two forms are caged together so close are their pairing and physiological affinities that no matter how small the cage they pair the same evening; the pairing, as is usual in the genus, lasts for a few minutes. Eggs are deposited the following night and three days later betray their fertility by changing from green to pink. The percentage of fertile eggs does not deviate from that of the pure species, and the same holds true of their successful hibernation and subsequent hatching in spring.

The latter operation commences between the periods of the early hatching *filigrammaria* and the later *autumnata* but always inclining distinctly towards the latter. This curious fact is important inasmuch as it is apparent that the male parent is exhibiting the greater influence, not, as Toyama¹ found in the silk moth, *Bombyx mori*, the female. Opposed as this observation is to Toyama's results, nevertheless it agrees completely with those in my reciprocal crosses between univoltine and bivoltine races of *Selenia bilunaria*, in which the male habit determined the fate of the ova. Precise dates of emergence are of little value, so greatly do they oscillate from year to year according to the forwardness of the season; in 1918, for example, *O. filigrammaria* hatched on February 28th, *autumnata* on March 18th and the hybrid ova on March 14th; in contrast with this today, March 15th, 1919, not a single ovum of any of the species or hybrids evinces the slightest sign of movement.

As they hatched the larvae were supplied with hawthorn (*Crataegus oxyacantha*); this they accepted and fed on with such avidity that they were in the ground early in April, that is, earlier than *autumnata* and

¹ Toyama, "On the Hybridology of the Silk-worm," *Rept. Seric. Ass. Japan* (1906).
Toyama, "On Certain Characters of the Silk-worm which are apparently non-Mendelian,"
Biol. Centrbl. Vol. xxxii. (1912).

later than *filigrammaria*. After allowing a reasonable time to elapse I removed the pupae from the cages and deposited them for aestivation in as natural a place as possible out of doors. They passed the summer without loss and appeared in due course, their first representative emerging seven days later than the earliest *filigrammaria* and ten days earlier than the first *autumnata*; in general it may be said that their whole period of emergence formed a perfect transition between those of the parental subspecies.

Description of Larvae.

As in *filigrammaria* the head was a dull smoky green. The ground colour was an olive green just intermediate to the blackish green of *filigrammaria* and the bright apple green of *autumnata*. The usual three white and yellow longitudinal stripes were present but their strengths varied, and agreed with those of neither parent. In *filigrammaria* the order of the strengths of the stripes is (1) Subdorsal, (2) Spiracular, (3) Supraspiracular; in *autumnata* this is (1) Spiracular, (2) Subdorsal and Supraspiracular equal; in the F_1 larvae the order was (1) Spiracular, (2) Subdorsal, (3) Supraspiracular—an evident compromise.

Ventrally, except that its ground is green, the markings strongly suggest *filigrammaria*.

On the whole the larva is clearly an intermediate to the dusky green *filigrammaria* with its violent contrasts and the more subdued lighter green *autumnata* with its less distinct stripes.

Pupae.

No obvious differences exist between those of *autumnata* and *filigrammaria*; naturally therefore none exist between either and the F_1 lot. Only one point needs to be emphasised and that is that there is no lack of pupal viability shown, such as occurs in the F_1 generation of the *O. autumnata* ♂ \times *O. dilutata* ♀ cross.

Imagines.

In making the original pairing a *filigrammaria* female with the two central bars as well as that preceding the subterminal pale line well developed was chosen, and paired with the usual pale grey pinewood *autumnata* male with much weakened central bands and almost obsolete basal lines, clouds and presubterminal band. From these parents I reared what at first sight seems a nondescript brood quite incapable of classification. Putting it alongside its cousins of both parent species broad differences are obvious. The general impression gained from the massed broods is that the F_1 insect as a whole is (1) less silky than

filigrammaria and (2) is of an ochreous grey tone entirely different from the monotonous quaker grey of *autumnata* or the violently chequered mass of *filigrammaria* where the powerful banding (of the deepest chocolate or carob brown obtainable) almost overpowers the dark fuscous shading of the ground and its pure white wedge-like interruptions. The significance of this will be grasped at once when attention is drawn to the fact that colour differences in these insects are not qualitative but are correlated with the quantity of pigment present. In *filigrammaria* the brown pigment is in excess; therefore it appears a photographic black; in *autumnata* it is slight and mixed with silvery white and hence looks grey; whilst in the hybrid it is medium so we get the impression of ochreous.

But, as with their cousins, a considerable range of fluctuating variation is observable in the vigour of the banding. Some, in the two central bands, are quite as clearly marked as *filigrammaria*, but this is counteracted by a lack of vigour in the presubterminal bar which combines the obsolescence of the corresponding *autumnata* band and the strength of that of *filigrammaria*. This, in combination with the lack of silkiness in tone, will always prevent one's confusing these insects with pure *filigrammaria*, in spite of their possessing the white wedges regularly outlining the cell in *filigrammaria* and occasionally perceptible on the nervures. Corroborative evidence too can be gleaned from the hindwings; on these *filigrammaria* possesses a well marked presubterminal band; *autumnata* displays a feeble one with a stronger terminal suffusion; the hybrid compromises.

From these heavily banded types we pass by degrees to insects exhibiting the whole range of variation in *autumnata* grey ground, in union with every degree of depth in the *filigrammaria* type of banding, so that in the end we reach, especially in the male, insects appearing at first sight indistinguishable from pure *autumnata* except in size. For the most part these betray their mixed blood not only in size but, on careful examination, in the exaggerated strength of the presubterminal band when compared with *autumnata*; if they do not, and the cases are few, the clear white wedges of *filigrammaria* serve to indicate their hybrid origin. But, whilst in all cases at least one of these points serves to direct one's judgment, in many of the most *autumnata*-like individuals both features appear to help one.

In connection with the relative size of the sexes the F_1 insect likewise seems intermediate. In *filigrammaria* the female size is 88.7% of that of the male, in *autumnata* it is 90.7% and in the F_1 insects 89.8%.

To sum up, as in size so in ground colour, markings, etc. both sexes, when due cognisance is taken of their fluctuating variation, and that of the parental forms, are clearly intermediate. But in spite of this I have not the slightest doubt that if captured wild about 80 % would be regarded as *autumnata* and 20 % as *filigrammaria* simply because, on the one hand, *autumnata* in its local races presents great variability in its banding, whilst on the other, *filigrammaria* nearly always appears in a heavily marked banded guise.

Genitalia.

The parent forms are too nearly alike (more particularly in the females) in this respect for great differences to be seen in the F_1 insects. Perhaps in the males the octavals are nearer than in *autumnata* and without doubt there are more bristles on the cristae.

Fertility of the Imagines.

In accordance with one's expectation in dealing with hybrids between parents so closely allied physiologically both sexes of the F_1 insects were fully fertile either when paired *inter se* or crossed back on *O. autumnata* or *O. filigrammaria*. All of the possible matings were made, and all of the ova deposited were fertile to the extent of 100 %. From these the F_2 , $F_1 \text{ ♂} \times \text{filigrammaria } \text{♀}$, *filigrammaria* $\text{♂} \times F_1 \text{ ♀}$, *autumnata* $\text{♂} \times F_1 \text{ ♀}$ lots were reared, those of the $F_1 \text{ ♂} \times \text{autumnata } \text{♀}$ being discarded owing to lack of cage room.

Like their parents the larvae of all these crosses were offered hawthorn, and like their parents they accepted it and fed up with trifling loss.

The F_2 generation.

The ova from the pairing $F_1 \text{ ♂}$ and $F_1 \text{ ♀}$ hibernated as such and commenced to hatch just prior to *autumnata* (March 10, 1918). As in the F_1 generation, the imagines obtained appeared so as to overlap the periods of *autumnata* and *filigrammaria* and thus accompanied their relatives in a new F_1 lot reared alongside them.

Of this generation very little can be said except that in every characteristic, size, relative sizes of male and female ($\text{♀ size} = 89.8 \% \text{ ♂}$), wing markings, and so on they were like the F_1 brood from which they were bred; they were intermediate to the two pure species. In no way was segregation in F_1 gametogenesis, and subsequent recombination in the F_2 zygotes indicated by the reappearance of grandparental characters. In size this feature was displayed to a marked degree as we shall see

later. Although the variation in wing pattern cannot be studied statistically and therefore so accurately as size, the degree of variation was obviously less than in the F_1 lot, for the extreme banded forms of that generation showing some approach to *filigrammaria* were non-existent.

Now it has become the fashion to explain the absence of any appearance of segregation of the grandparental form in cases like this on the ground that in place of few clear cut unit characters depending on similarly definite genes we are dealing with hosts of factors segregating and recombining independently, some with and some without dominancy. Under these circumstances, it is urged, it would be futile except in thousands, perhaps millions, of cases to expect a perfect grandparental segregate; what we are to look for, we are told, is a smooth variational curve of more extended spread than in the F_1 brood. But we have pointed out that the variation exhibited is most markedly less than that shown by the F_1 insects, which cannot be explained on other grounds than the ordinary fluctuating and continuous variation due to the play of the more or less plastic germinal material of the pure species. Why then should we invoke multiple factors to explain F_2 variation and not that in F_1 ? To drag in two distinct explanations for one and the same phenomenon seems absurd.

The balance of probability lies in favour of the view that we are rather concerned with contamination of the gametes, or with permanent gametic blending; this I strongly suspect is the rule in interracial and interspecific crosses, if any weight at all attaches to their generally uniform evidence.

Again the insects proved completely fertile in both sexes and a number were confined for ova; these were successfully obtained and hatched in due season in the following year.

The F_3 generation.

As ova (hatched March 8, 1918) and pupae this generation offered no obvious peculiarities, but the case was different with the larvae, which in about one half of the instances tended to exhibit a massing of the darker dorsal suffusions on each side of the paler lines edging the dorsal vessel, particularly just before the anterior trapezoidal tubercles, in this fashion developing a pattern involving a series of dorsal lozenges. This was unusual enough for me to select a single larva for description but still not extraordinary enough to be considered beyond the possible range of variation. Nevertheless, in the light of subsequent knowledge, it was

evidently correlated with the advent of a previously unknown imaginal form which we shall discuss below.

The larvae fed up normally and the pupae passed the summer safely to yield a total of 86 imagines in autumn. These divided themselves sharply into two sections, one composed of 27 males and 8 females being precisely like their parents and therefore intermediate between true *autumnata* and *filigrammaria*. This intermediate portion needs no description and would not be further mentioned except to indicate that it included one teratological female in which the left antenna was duplicated. The other division composed of 24 males and 27 females was wholly dissimilar; *the individuals in it resembled neither their parents nor, save in a manner common to the genus, did they approach the pure species from which they had been derived.* Moreover, although fluctuating variation was present, still the impression one gained from the series was one of complete uniformity. In view of their peculiarities therefore they are worthy of a detailed description.

The ground colour is of an almost pure white interspersed with a few grey scales; in two cases, one male strongly and one female weakly, this is suffused with a smoky tinge. 'Across this ground passes a band differing widely in its build from that seen either in *autumnata* or *filigrammaria*—or even in *dilutata*. In ordinary pure species we have two central bands, each of which is composed of two lines more or less parallel, connected up by a suffusion varying in depth with the species and with the genetical type of the race within the species. In the *filigrammaria* female employed to produce the F_1 generation, the shading was so deep as to differ in no wise in intensity from that of the bounding lines; on the other hand, in the *autumnata* parent this was very much less concentrated. As we have seen the F_1 , F_2 , and a portion of the F_3 lots were intermediate in this respect. The band in the central area of the aberrant section is wholly distinct in design from any of these. Almost in every case the bounding lines of the bars in which they occur are distinct enough, although they give one a sense of being out of focus, but, what is more important, the outer member of the inner band is obsolete in all cases; in others (all males) the same fate has befallen the inner one of the outer band. In the latter event, the two existing lines tend to be united by a brownish suffusion and in all the examples the two normal outer ones show the same trend. Occasionally (to be exact in half a dozen examples), the line proper to the outer band moves toward the base of the wing and becomes central; here, likewise, a brownish suffusion tends to occupy the whole of the central wing area. Another curious

feature lies in the fact that the outermost line of all, where it intersects the sixth nervure, projects along it and causes the whole band to jut out at that point. Outward from the central band is a broadish white bar traversed by a wavy line; this, again, is much clearer than in any form previously considered. It is succeeded by an almost solid blackish suffusion in which the pale subterminal line is wholly or partially buried. Within the limits of this suffusion the nervures are heavily outlined in a perfect manner quite foreign to *filigrammaria* or *autumnata* or their other hybrids. A similar encroachment of the black scales outlining the nervures of the cell and those issuing from it is made along other nerves, so that in extreme cases the whole nervure system is lined in black. At the same time a change in the relative position of the discoidal point is seen. Normally it lies in the angle of the elbowed line of the outer band. Here, in most examples, it lies on that line and even on others beyond it, a displacement never encountered in *autumnata* and *filigrammaria*. The total effect of this change in "band formula" is to throw the band into a condition recalling the narrowed complete carpet band observable in so many genera of the Larentiad group—even those removed fairly widely from *Oporabia*—and possibly, therefore, indicating that the completion of the band is reversional.

On the hindwings the terminal bands and suffusions are obsolescent but the increased strength of the black scaling outlining the nervures is very marked: in particular, the black V at the base of veins 3 and 4 in the forewings, just at their origin in the cell, is reproduced as one never sees it elsewhere.

What is the cause of this postponed or pseudo-segregation? Had it occurred in such forms as *Oenothera Lamarckiana* it would have been heralded as a genuine example of mutation. This explanation, however, is here excluded because the combinations which were used to synthesise the form are known. Although two generations removed, the original parents were hybrid in origin and the phenomenon, whatever it is, is one of hybridity, and its explanation must lie in some germinal disturbance brought about by that—and germinal irregularities were met with early. As will appear in my subsequent paper on the gametogenesis of these hybrids the chromosome complement of *filigrammaria* is 37 and that of *autumnata* 38—these being the haploid numbers. So nearly homologous are they that, in the gametogenesis of the F_1 hybrids, in many cases all of the *filigrammaria* chromosomes find mates; in other cases up to four do not, with the consequence that certain oocytes and spermatocytes are endowed with 38, 39, 40, 41, 42 or 43 chromo-

somes respectively, so that in forming the F_2 generation a combination of any two of these takes place. Thus the F_2 zygotes commence life with a mechanism intended to deal with 76 chromosomes at the most, in place of which 86 may occur in each nucleus. Consequently, in their gametogenesis, in addition to failures in homology, merely mechanical complications ensue ending not only in chromosome disturbance but in their actual loss and degeneration in the cytoplasm. In this case, if the chromosomes be in very truth the heredity bearers, then there cannot but be a manifestation of new characters or combinations in the imagines. The great uniformity of the F_3 pseudo-mutants, however, suggests that if this explanation is, as is almost certainly the case, true, then there must be some preferential setting up of some individual type of chromosome combination. Still it is quite possible that the other types may not render the zygotes possessing them in any way worthy of special note.

To my mind the importance of this result is very great; the parallel nature of the occurrence with those secured by De Vries and others in *Oenothera* suggests just as forcibly to me, as did other considerations to Bateson, that *Oenothera Lamarckiana* was and is not a pure type, and not being pure is no fit subject for ordinary genetical experiment, especially when no one has the faintest knowledge of its actual genotypic composition. Much less then can it be held a fit subject upon which to base such a fundamental doctrine as mutation. In thus pointing out the views I have been led to adopt concerning *Oenothera* I do not wish to deny that mutation occurs: that it does we have ample evidence in our own genus in the *latifasciata* aberrations of both *autumnata*, *filigrammaria* and *dilutata*, not to mention other well-known cases both in domesticated and wild animals and plants.

The Back Cross between the F_1 ♀ and O. autumnata ♂.

This was reared from a pairing of an *autumnata* male of the feebly marked, suffused pinewood form and a medium female from the F_1 lot. Ova were successfully obtained and wintered safely to emerge just prior to *autumnata* in the succeeding spring. The larvae revelled in the hawthorn they were offered and fed up rapidly without loss.

Of the larvae and pupae little can be said save that almost no signs of *filigrammaria* influence were discernible unless they lay in the slightest possible strengthening of the longitudinal stripes, the darker ground of head and body in *filigrammaria* failing to show any effect.

In the imagines (save in size) one looks likewise in vain for any

indication of *filigrammaria* blood; their markings are absolutely those of typical *autumnata*. But they possess this very important feature; their range of fluctuating variability is down to a minimum, for I have never possessed such a uniform brood from any female in the genus. They give one the impression of a deadly uninteresting monotony not even relieved by the usual sexual dimorphism of the group.

In variation exhibited they are far inferior to either pure *autumnata* or to *filigrammaria*, whereas if the multiple factor theory be the genuine explanation of the continuance of the F_1 blend here as well as in the F_2 lot, the range ought to have been much greater rather than much less. The independent segregation of all of many unit factors in the oogenesis of the F_1 female should have resulted in an immense field of variation when in zygotic combination with the gametes of the *autumnata* male. Again the evidence of the cross distinctly opposes the multiple factor scheme and its gametic purity; it is all in favour of lasting gametic contamination or blending.

It will thus be seen that a single back cross has sufficed, to all intents and purposes, to cause the hybrid to revert to one of the parent species.

As in other cases the hybrid insects were fertile in all directions but only larvae from one mating in which they took part were reared to maturity; these were the result of pairing the insects *inter se*. So alike were the insects involved that no selection of genotypes was possible. To ensure as variable a progeny as possible ten females, chosen at random, were caged up with a similar lot of males, and about a tenth of the mixed ova retained for breeding. In spite of these precautions, the insects bred showed no signs of segregation and in fact were indistinguishable in size or design from their parents and, furthermore, displayed the same limited extent of variation.

The Back Cross between autumnata ♀ and the F_1 ♂.

Owing to a shortage of cage room this cross was not proceeded with in 1918. During that season, however, a further F_1 brood derived from the same stocks as those employed in the 1917 experiments was reared. From this brood an average male was taken and paired with a female *autumnata* from the same strain as utilised before. Fertile ova were duly obtained, and the larvae issuing from them reared during the present season.

Up to the present (October 23rd, 1919) two-thirds of the pupae have yielded their imagines. These are of the same expressionless type

as those of the preceding cross and therefore display a very contracted range of variation. Once again a single back cross has apparently sufficed to restore the *autumnata* facies, and once again the evidence gained points to permanent gametic contamination.

The Back Cross between the F_1 ♀ and filigrammaria ♂.

Here a barred *filigrammaria* male such as was employed originally was paired with an F_1 female. The early stages need no special treatment but one must not omit to state that the larva leant very distinctly toward *filigrammaria* in the strength of its stripes.

The imagines obtained, if they had been captured in nature, would undoubtedly have been regarded as *filigrammaria*, but nevertheless direct comparison with both *autumnata* and *filigrammaria* shows that they present clearly enough points of divergence which we shall proceed to point out.

Before doing so, it must be noted that their range of variation is, unlike that of the last cross, of the same extent as in the parents but emphatically not greater.

The first great difference between it and *filigrammaria* is the superior size, the mean being in both sexes slightly higher than that of the F_1 insect instead of less, a circumstance brought about by the undue weight of one of the superior size classes which two facts prove to be of no definite import: (1) in the female one of the lower classes is similarly overweighted but not enough to counterbalance the former; (2) the reciprocal cross shows no such features. The only other sign of the influence of *autumnata* worthy of special note is that, whilst every phase of variation of pure *filigrammaria* is encountered it is always toned down, no matter how small the degree, by the indecision introduced from *autumnata*.

To sum up, the insect comes very near to *filigrammaria* but shows very obviously its *autumnata* blood and is thus a good intermediate to the F_1 lot and *filigrammaria*. Thus we perceive that one back cross in this direction does not suffice to cause a reversion to the parent type. This being so, and the insect being perfectly fertile, it was once more crossed back on *filigrammaria*, females of this stock being paired with males of *filigrammaria*.

The Back Cross between the preceding and filigrammaria ♂.

The larvae of this cross were exactly the same as their cousins of pure *filigrammaria* blood which were reared in the adjoining cage. And the same remark is applicable to the imagines; of all the large number

reared only four would be regarded as other than pure *filigrammaria*, not even a specialist being able to detect the faintest indications of *autumnata*. In the case of *filigrammaria* it has required two back crosses to secure the passage back to that species.

As for the four aberrant individuals they show very considerable approach to the pseudo-mutants of the F_3 generation; in fact, one of them is precisely the same as those, and the other three only differ in being decidedly darker. The same consideration of mitotic dislocation is, in all probability, responsible for their appearance, because again in the gametogenesis of the back cross between F_1 ♀ and *filigrammaria* ♂ we are dealing with a second hybrid generation; the same interference as we considered possible in the case of the F_2 generation may be at work here. If this be the case a preferential setting up of a definite type of gamete, when such are aberrant, may conceivably result in similar phenotypic if not genotypic manifestations.

The Back Cross between F_1 ♂ and filigrammaria ♀.

This generation, except that we have the expected drop in mean size toward *filigrammaria*, agrees with that obtained in the reciprocal combination and cannot by any test save this one of size, which is certainly a matter of chance, be separated from them. Once more a single back cross has failed to restore the *filigrammaria* condition. The very

TABLE V.

Summary of sex ratios in the Oporabia autumnata-filigrammaria experiments.

Culture	Males	Females
<i>Oporabia autumnata</i> from Pinewood	81	63
<i>Oporabia autumnata</i> from Birchwood	48	30
<i>Oporabia filigrammaria</i>	90	106
F_1 <i>Oporabia autumnata</i> ♂ (from pine) × <i>O. filigrammaria</i> ♀ ...	19	60
F_1 <i>Oporabia autumnata</i> ♂ (from birch) × <i>O. filigrammaria</i> ♀ ...	33	30
F_2 <i>Oporabia autumnata</i> ♂ (from pine) × <i>O. filigrammaria</i> ♀ ...	51	31
F_3 <i>Oporabia autumnata</i> ♂ (from pine) × <i>O. filigrammaria</i> ♀ ...	51	35
Back cross 1 = <i>Oporabia autumnata</i> (pine) ♂ × F_1 ♀ (pine) ...	51	51
Back cross 2 = <i>Oporabia filigrammaria</i> ♂ × F_1 ♀ (pine) ...	30	31
Back cross 3 = F_1 ♂ (pine) × <i>Oporabia filigrammaria</i> ♀ ...	17	15
Back cross 4 = <i>Oporabia filigrammaria</i> ♂ × Back cross 2 ♀ ...	41	49

obviously superior power of *autumnata* in this respect is very curious. One would have thought that the heavier barring in a palpable case of blending inheritance would have had a greater effect than the feebler state of *autumnata* in this respect, and that therefore there should have been a speedier reversion to *filigrammaria*. But one must not forget that *autumnata* is the phylogenetically older form and that *filigram-*

maria is derived from it, the anomaly may therefore be ancestral in cause.

The F_1 generation of the cross between *autumnata* ♂ and *filigrammaria* ♀, the *autumnata* being of the pale birch type.

This calls for no special remark; except in the absence of suffusion, the brood recalls in every way that of similar origin when the birch male was replaced by a pinewood male.

(2) *Hybrids between Oporabia autumnata and O. dilutata.*

Immediately after my discovery of *Oporabia autumnata* in Wilton pinewood I made preparations for securing the two possible crosses between it and *O. dilutata* and to that end reared a large number of pupae of both species which emerged in due time. Unfortunately I had not then realised the highly specialised nature of the pinewood race of *O. autumnata* in respect to its time of emergence, for I had conceived the idea that in dealing with great numbers some few at least would appear simultaneously with *O. dilutata* in October. Such an event, however, did not happen; without a single exception the *autum-*

TABLE VI.

Summary of sex ratios in the Oporabia autumnata-dilutata experiments.

Culture	Males	Females
<i>Oporabia dilutata</i> . Totals of wild stocks from which parents of hybrids were chosen	77	72
<i>Oporabia autumnata</i> (birch). Totals of three broods from which parents of hybrids were chosen	85	69
F_1 <i>Oporabia dilutata</i> ♀ × <i>O. autumnata</i> ♀ (3 broods) ...	52	47 (no ovaries)
F_1 <i>Oporabia autumnata</i> ♂ × <i>O. dilutata</i> ♀ (6 broods) ...	6	0 ¹

nata had long since ceased to emerge when my first *dilutata* came out. The experiment was therefore a failure. Profiting in the succeeding season from this experience I beat a considerable quantity of wild *autumnata* larvae in addition to hosts of *dilutata* from diverse sources and once again provided myself with an abundance of pupae of both species. These preparations ended in complete success for when the first *dilutata* appeared in October I had both sexes of *autumnata* caged up. Placing a melanic *dilutata* female in a cage with *autumnata* males and *vice versa*, I confidently awaited results. Almost at once I had the pleasure of seeing ova deposited, and these in every case changed

¹ This result was obtained from over 400 pupae—all males—but which nearly all died as is one's usual experience in this cross. I have reared single females from this crossing in three different broods (otherwise male) in different years; they were indistinguishable from pure *O. autumnata* females.

colour, those from *dilutata* female to a dull ruby and those from *autumnata* females to salmon pink. It will thus be evident that cross fertilisation in no wise altered the colour of the fertile ovum of any given female from what it would have assumed on ordinary fertilisation. No acceleration in emergence due to hybridity was manifested, and in both instances the ova hatched in the April of the following year, when practically every ovum yielded a larva.

The larvae were offered hawthorn (*Crataegus oxyacantha*) and displayed no hesitation in accepting it. In habits both sets resembled *autumnata*, for they fed ravenously and showed neither wandering tendencies nor slowness in feeding up. No tangible differences¹ existed between the two hybrid larvae and both are lovely creatures, only one larva in the group, that of the hybrid between *dilutata* female and the *F*₂ *filigrammaria* ♀ × *autumnata* ♂, surpassing them in beauty of design. They possessed the pleasing soft apple green ground colour of *autumnata* with its more or less conspicuous yellow longitudinal stripes, and upon it were superimposed the reddish dorsal blotches of *dilutata*, toned down here to a delicate ferruginous red. They pupated, one and all, in early June. Since events in the two reciprocal crosses pursue different courses after pupation it is best to consider the two life histories subsequent to that event separately.

The F₁ generation of the cross between O. autumnata ♀ and O. dilutata ♂.

Before passing on, however, attention must be drawn to extraordinary abnormalities in the behaviour of certain hybrid larvae, resulting from the cross between *Oporabia autumnata* ♀ and *O. dilutata* ♂, in their penultimate instar. About fourteen of these, instead of moulting normally, span a silken pad and thus attached themselves at the end of March to twigs of the food plant where they remained for over a fortnight without making the slightest endeavour to cast their skins—an event usually occurring two or three days after cessation from feeding. Without considering further possibilities I at once conceived the notion that they were attempting to resume some long abandoned habit of larval hibernation, and therefore removed most of them with the full grown larvae to the cages used for pupation; here, owing to their being overwhelmed by the random silken threads spun by larvae seeking

¹ Save in size in the first instar; as the cubical contents of an ovum of *dilutata* are less than two-thirds of those of an *autumnata* ovum any larva, hybrid or otherwise, produced from the latter is bound to be larger than one issuing from the former.

to enter the soil, they died. Luckily, I had taken the precaution of isolating one specimen to determine exactly what course it would pursue. For five weeks it remained motionless, and then it assumed a very unhealthy colour suggesting that it was moribund. Clearly, however, this colour change was only preparatory to exuviation, for when I looked at it later I discovered that it had moulted and had yielded, not a fifth stage larva, but a strange monstrosity, larval in colouration and semi-imaginal in structure. At first sight its anomalies seemed to exist chiefly in the pairs of unusual appendages borne by the meso- and metathorax, these evidently being external wing buds exactly comparable with those seen in heterometabolous forms like the Orthoptera. More minute inspection showed many further approximations to imaginal conditions which were more particularly visible on the structures appertaining to the head.

In size the head was much inferior to that of the last stage larva, and in this and in shape it agreed more nearly with the imago. Nor was the resemblance confined to size and shape, for the antennae were much longer than the ordinary larval type and possessed numerous joints, it being impossible to determine the exact number owing to their being fused; in shape they closely resembled an inverted Indian club. The eyes, moreover, partook of the characteristics of both larva and imago, for the imaginal compound eye was represented by rounded projections of normal size, more or less regularly reticulated but not developing regular facets; on the right member of these eye masses appeared two ocelli with rudiments of others and on the other one ocellus with rudiments.

The larval jaws were present and, as a matter of fact, apparently duplicated, but this I suspect to be due to imperfect moulting of the earlier pair.

The external wing rudiments were not all of equal length, that on the right of the mesothorax being 3 mm. long and the other three roughly 1.75 mm.; all possessed vestiges of the usual imaginal neurulation.

The legs, whilst in the main larval, are difficult to describe owing to the presence of obvious traces of imaginal structures. Although perhaps a little flattened, the prolegs do not call for special treatment.

The advent of external wing buds in a holometabolous insect is capable of two explanations; either we are dealing with atavism, in which case we are receiving glimpses of the course matters took long ago in the evolution of winged imagines from original larval forms, or we are concerned with, on a large scale, the phenomenon of anticipation

noted in minor characters by Denso¹ and myself² in larvae of Sphingid and Bistonine hybrids. In my opinion the latter, from the evidence presented, is the correct view, i.e. that we have a precocious development of imaginal organs induced by the disturbing influence of hybridity.

Rare as this occurrence is, it is not unknown even in pure species, although as far as the Lepidoptera are concerned only one previous case is on record, that of larvae of *Bombyx mori* reared by Cesare Majoli in 1813; these were in every way similar to my *Oporabia*. In the Coleoptera—still holometabolous but not so complex in the mode of origin of its wings—other cases are known; in this group it is not so wonderful, for the invagination of the hypodermal cells destined to give rise to the wing buds is of a much simpler type, and very little displacement would cause their appearance externally. To the best of my knowledge the recorded instances in the beetles relate to the common *Tenebrio molitor*, and to *Anthrenus varius* and *Dendroides canadensis*. Our knowledge of the first case is the most extensive, for Prof. Heymons³, after his discovery of the first larva, was able to find others and give them detailed study. Busck⁴ added *Anthrenus varius* and Riley⁵ *Dendroides canadensis* to the meagre list of species displaying this abnormality. Making due allowance for the difference in order, these coleopterous freaks do not differ widely either from the conditions of Majoli's silkworms or of my *Oporabia* and are therefore directly comparable with them.

We shall now return to the fate of the larvae pupating normally. Anticipating as usual an aestivation of some four months I allowed the pupae to harden for a fortnight or so and then proceeded to dig them up in order to store them under natural conditions. When I examined the cage for this purpose sixteen days after pupation, I was greatly astonished to find that a female had already emerged, and this observation caused me to scrutinise the pupae daily. And this proved a very fortunate thing, for during the next few days approximately one half of my stock emerged and every individual doing so proved to be a female.

¹ Denso, "Die Erscheinung der Anticipation in der ontogenetischen Entwicklung hybrider Schmetterlingsraupen," *Zeit. für Insektenbiol.* iv. (xiii.) pp. 128—135, 170—176, 201—208 (1908).

² Harrison, "Studies in the Hybrid Bistoninae," *Journal of Genetics*, Vol. vi. No. 2, p. 102 (1916).

³ Heymons, "Flügelbildung bei der Larve von *Tenebrio molitor*," *Sitz. Ber. Ges. Nat. Freunde*, pp. 142—144 (1896).

⁴ Busck, "Larvae of *Anthrenus varius* showing wing pads," *Proc. Ent. Soc. Wash.* Vol. iv. p. 123 (1897).

⁵ Riley, "The abnormal appearance of external wingbuds in larvae of holometabolous insects," *Ent. News*, Vol. xix. No. 9, pp. 136—137 (1908).

After waiting in vain six weeks for further emergences I unearthed the remainder of the pupae expecting them to be dead, but was astounded to discover that practically every pupa was alive and healthy, and that every one was a male. These were packed as usual in moss and coconut fibre in air-tight tin boxes and laid aside to be carefully inspected at intervals. No further moths put in their appearance until October, when the rest came out in company with the parent species.

Description and discussion of the resulting insects are greatly complicated by my unfortunate but unavoidable choice of melanic *dilutata*, which at the time was the sole form of the insect accessible to me. Owing to the blurring effect of the melanism other details are rendered obscure. Very obviously, however, the males are strict intermediates when correct value is attached to every point, whilst just as certainly one would assign the females to pure *dilutata*. Here then was the first hint of that sex-linkage in inheritance in the genus which I have pursued at some length above. Subsequently, in order to clear away the indecision induced by the melanism I have employed ordinary birchwood *autumnata* and non-melanic Irish *dilutata* in my later experiments, so that more exact comparison of the hybrid insects with their parents becomes possible.

In the light of these new broods I find but little to alter in my views; the male remains intermediate no matter what strain of *autumnata* is involved (and I have now succeeded in introducing all of the races known to me from birch, alder and pine); still I must confess that the closeness of the females to pure *dilutata* is not so decided, for on the clearer non-melanic ground colours *autumnata* shows some, if varying, influence.

Full description of all of the imagines reared in the later trials would be impossible, such a wide range of variation is introduced by the continuous or fluctuating variation so prominent in both parent species. Due to this fluctuating variation in the males, one can almost say that a perfect transition exists from pure *autumnata* to pure *dilutata* in outward appearance. In spite of this their hybrid nature is at once betrayed to the expert by the peculiar texture of the wing scaling, which in every case compromises between the coarse whity-yellow tone of *dilutata* and the fine silkiness of *autumnata*, and gives us a silvery grey ground quite unlike what one encounters elsewhere in the genus, but still recalling that exhibited by several other Larentiad species. One very important point must be singled out for special mention and that is that if the clear, sharply marked birchwood *autumnata* with the

silvery white central area provides the male, then that pattern is dominant, and the insect obtained differs in no respect from *autumnata* save in its cool grey ground. Similarly certain of the other *autumnata* patterns, for instance that of the distinctly barred *sandbergi*, show like tendencies but their dominance is never complete.

In the female the fluctuating variation is likewise great, although, as I have indicated previously, but rarely would this sex be deemed other than pure *dilutata* whether they were melanic or non-melanic—a fact that would not prevent the eye of the trained observer from detecting differences in many cases.

The indications of other structural characters are quite in harmony with those of the wing markings. The male antennae are absolutely intermediate between the finely jointed antennae of *autumnata* and the much coarser ones of *dilutata*, and the genitalia simply continue the tale. The hook on the valve is developed but remains quite small, and the head of the labides is intermediate in size. So, too, in the female the signa of the bursa copulatrix (Figs. 1, 2, 3, 4) on the whole favour *dilutata*, but some hint of *autumnata* may be gleaned from the sculpturing of the upper one.

In making the necessary dissections for the study of the female genitalia a very important feature was revealed; that the ovaries of the insect were lacking. And herein lies the explanation of the precocious appearance of the females, the two factors of emergence and completed gametogenesis being so correlated that the female leaves the pupa soon after the operation of oogenesis is finished. No oogenesis being possible with this hybrid, the insect develops and emerges at once. That delayed gametogenesis accompanies delayed emergence I can readily prove. In 1917 I had two lots of *dilutata* pupae, one of which had contracted a bacterial disease during the last larval skin which in many cases did not prove fatal; the other lot was not infected. Both lots were freely drawn on for material for cytological work, on the assumption that larvae which had successfully pupated could not be diseased. When microscopical preparations of the gonads were made it was found that in the healthy lot the maturation divisions were completed by the first week in September, whilst in the other the process had barely commenced at the end of the month. Then every germ cell, from oogonium to ovum, from spermatogonium to spermatozoon, was crowded with bacteria.

During that season the first wild imago of *dilutata* was seen in the woods on September 24th, and for practical purposes the species was over by October 13th, when on my searching for wild females for stock only

worn examples of that sex were captured. Roughly, the same period covered the emergence of my sound domesticated brood; on the contrary, the bacteria infected insects failed to show themselves until October 15th and straggled on, most unusually, until the middle of November. This *dilutata* brood was not alone in thus exhibiting the phenomena of infection and retardation, for both of the *dilutata-autumnata* crosses of that year likewise contracted the disease, and they too were late in emerging. Despite the infection, as is customary, the females of the *autumnata* ♀ × *dilutata* ♂ lot were obtained in June and July, proving thereby that the presence of bacteria did not inhibit somatic development; on the other hand the males of both broods, as well as the odd female of the reciprocal cross, instead of accompanying the alderwood insects only started to emerge on October 13th, subsequent to which date they yielded small numbers over a protracted period.

From what has been related it is manifest that further work with the hybrid females was hopelessly excluded, but matters with the males were different. Genitalia, primary and secondary, external and internal were alike perfect, the testes containing an abundance of well developed spermatozoa. Arrangements were therefore made to confine the males in cages with *autumnata* and *dilutata* females to secure the apparently certain back crosses. But matters did not proceed smoothly; ova were not forthcoming immediately, and it was only after a fairly long interval had elapsed that eggs were obtained. In the two crosses no marked differences in the fertility of the ova were noticed, some 30% in both cases being fertile. Spring, however, told a different tale, for every ovum from the pairing between *autumnata* ♀ and the F_1 ♂ collapsed after development had been initiated, whilst every fertile egg in the cross between *dilutata* ♀ and F_1 ♂ delivered a sturdy little larva which fed up speedily and, until the last moult, well. These larvae, in direct opposition to their ostensible three-quarters *dilutata* blood, could neither in design nor habits be distinguished from those of the F_1 generation of the *autumnata-dilutata* crosses. There was not the slightest sign of passage toward *dilutata*—a totally different phenomenon from the almost instant return, on back crossing, of the F_1 *autumnata* × *filigrammaria* insects to their parent forms. This undoubtedly depends directly on the different courses pursued in the gametogenesis of the two F_1 broods. In the *filigrammaria* ♀ × *autumnata* ♂ cross nearly all (sometimes actually all) of the chromosomes find mates previous to the first maturation division, so that a reduction division does occur accompanied, in my opinion, with an “averaging up” of the values attachable to the various

genes. On the contrary, in the *autumnata* ♀ × *dilutata* ♂ F_1 lot no pairing of chromosomes takes place at all, and in consequence all of the maturation divisions are equational. Every gamete then derived from the *autumnata* ♀ × *dilutata* ♂ males is endowed with the sum of the haploid chromosome numbers of *autumnata* and *dilutata*, i.e. it has $30 + 38 (= 68)$. In other words it has a full complement of those from each parent. When such gametes encounter in fertilisation those of *dilutata* a zygote is generated the cells of which contain a chromosome number of $30 + 30 + 38 (= 98)$ —a double set of *dilutata* and a single set of *autumnata* chromosomes. Now if the chromosomes are the heredity bearers and the various unit factors are located in them then the genes from *dilutata* are duplicated. But Nilsson-Ehle¹ and Shull² have shown in their experiments on chaff colour in grain and capsule shape in *Capsella* that duplicate genes are not necessarily cumulative in effect; whence it follows that in back crosses such as these, where no genuine reduction division takes place in the F_1 insects, the phenotype of the back cross may not vary perceptibly from that of the F_1 insect. Furthermore, since in the gametogenesis of the back cross pairing of homologous chromosomes will take place, its gametes will be identically the same as those of the F_1 insect so that reversal to parental type can never be brought about in the case of hybrids with such a form of gametogenesis. No matter through how many generations we pursue the experiments the constitution of the genotype produced can never differ from that obtained in the first back cross; thus a constant intermediate form will be maintained.

Of the larvae only five pupated, bacterial disease carrying off the rest. All of these were males and this is precisely the condition of the *dilutata* ♀ × *autumnata* ♂ broods in general; still, in view of the small numbers involved no definite importance can be attached to the fact. Before emergence three died simply through lack of viability, and two began to develop but were sacrificed for cytological purposes. However, this back cross has once more been obtained and I hope to pursue my investigation with it during the present season.

¹ Nilsson-Ehle, "Einige Ergebnisse von Kreuzungen bei Hafer und Weizen," *Bot. Notiser* (1908). Nilsson-Ehle, "Kreuzungsuntersuchungen an Hafer und Weizen," *Lunds Univ. Årsskr.* N. S. Vol. v. pp. 1—122 (1908).

² Shull, "Duplicate Genes for Capsule Form in *Capsella bursa-pastoris*," *Zeitsch. für Abst. und Vererb.* Vol. xii. pp. 97—149 (1914).

The F_1 Generation of the Cross between dilutata ♀ and autumnata ♂.

Of this cross little can be said save that one must refer to its lack of pupal viability. If 2% of all of the pupae laid aside in July are bred then a remarkable feat has been performed—and in the genus one usually rears 75% as a minimum. In all of the almost countless numbers of larvae of this particular parentage I have reared, only once have I exceeded this figure of 2%, and that was in 1915 when I raised over 12%. The mortality always becomes evident just when the maturation divisions are proceeding and may be due to some reaction between somatic and germ cells but how brought about one can only guess. Obscure hints, nevertheless, may be gleaned that such is the case from the fact that often enough the act of pairing and deposition of hybrid spermatozoa in the bursae copulatrices in certain *Bistonine* females is instantaneously fatal.

The insects bred in the present cross are nearly always males, and in that sex they cannot be separated when in representative series from those secured in the reverse cross. But curiously enough, beginning with my first experiment when I reared one female and seven males, I have on three separate occasions bred single females. It must not be assumed that this is due to differential viability of the sexes, for it is not so. Examination of brood after brood of dead pupae will show that they consist wholly of males. The case therefore becomes parallel to those of the crosses between *Tephrosia bistortata* ♀ and *T. crepuscularia* ♂, *Nyssia zonaria* ♀ and *Lycia hirtaria* ♂, *Nyssia graecaria* ♀ and *Lycia hirtaria* ♂ which I have discussed in a recent paper (now in the press) entitled "Studies in the hybrid *Bistoninae*; iv. Concerning the Sex and Related Problems." In brief my explanation is that the failure of the females is brought about by a difference in potential in the male sex genes in the species involved in the crosses.

The genetical behaviour of the odd females has already been described in the paragraphs on sex-linked inheritance and thus needs no further treatment here. That of the male does not differ greatly from that of the same sex of the reciprocal hybrid. I have never dared to risk one of the very rare females in pairing this F_1 generation *inter se*, but I have made the two possible back crosses with *O. autumnata* and *O. dilutata* females. With *autumnata* females about 25% of the ova deposited were fertile and with *dilutata* about one half that. All wintered satisfactorily enough, but none of the latter hatched and only two-thirds of the former. Although those hatching seemed healthy enough, they

died one by one before revealing characters sufficiently striking to admit of study.

Whether by mere coincidence or founded on some genuine physiological basis, it is observable that in all four back crosses, when the female in the back cross is the same as that entering into the original cross, then no ova hatch; and, on the contrary, if the female of the back cross is of the same species as that providing the male in the first cross, then the eggs hatch and the larvae progress more or less favourably. That the matter is more than mere coincidence the separate and confirmatory evidence of several broods seems to show.

(3) *Miscellaneous Pairings.*

For an exceedingly long time I have striven to obtain the necessary pairings for introducing *O. dilutata* into the *autumnata-filigrammaria* series of hybrids, but only to be thwarted year after year by the exasperating way in which almost invariably *O. filigrammaria* and any hybrid in which it took part were over before *dilutata* appeared: the latter species is immovable in its preference for the last days of September and for October as the period of its imaginal activities. However, 1917 put me in possession of a brood of *O. dilutata* which produced a small proportion of its members in September and these most luckily coincided in their emergence with stragglers of several of my *autumnata-filigrammaria* broods as well as with belated examples of *filigrammaria* itself. I was therefore able to enclose *dilutata* females with F_1 (*filigrammaria* ♀ × *autumnata* ♂) males, F_1 (*filigrammaria* ♀ × *autumnata* ♂) females with *dilutata* males, *dilutata* females with F_2 males of the same cross as well as to attempt reciprocal crosses between *dilutata* and *filigrammaria*; what these matings produced will be discovered below.

The Pairing of dilutata ♀ and the F_1 (filigrammaria ♀ × autumnata ♂) Male.

In this cross over a hundred ova were laid, and although I did not observe the pairing such had evidently taken place, for out of 114 ova seventeen changed colour from green to pink four days after deposition. This proved their fertility but, nevertheless, when spring made its influence felt, instead of turning to the dark steel blue of ova developing normally they collapsed and assumed a rusty hue and produced no larvae.

The Reciprocal Pairing to this.

Pairs caged up simultaneously with the last lot yielded ova, none of which changed colour. They were thus infertile—a fact that might

have been brought about either by a lack of physiological affinity between the possible parents or by a failure to copulate, the former being the more likely explanation, so readily do the *Oporabia* species cross pair.

The Cross between dilutata ♀ and the F₂ Male.

This mating proved, for this set of experiments, a very conspicuous success, for no fewer than 73 fertile ova were laid in the interstices of the muslin cage and hatched in March 1918. I offered them the usual pabulum employed by me, i.e. *Crataegus oxyacantha*, and this, as with all other *Oporabia*, hybrid or pure, was instantly accepted. All of the little larvae reached the second instar, when I began to lose a few. Still, in the end, 46 pupated.

These larvae were gorgeous creatures and far surpassed the magnificent caterpillars of the two *dilutata-autumnata* crosses. They possessed the beautiful green ground with the yellow bands and dorsal red blotches of those forms combined with a darker, olive green shading derived from *filigrammaria*—the whole scheme of colouration contrasting so as to yield a very striking effect. In the possession of this rich garb the larvae showed the presence of the three contributing forms *autumnata*, *filigrammaria* and *dilutata*.

They went to earth and spun cocoons in no wise unlike those of their congeners, and every single larva produced a fine healthy pupa which remained alive until late in August when the maturation divisions ought to have been proceeding. Then the fatal want of viability so often the bane of hybrid pupae in the production of which *dilutata* females have taken part exerted its influence, and every pupa died, just as is one's experience, with but rare exceptions, in the crosses referred to. Fortunately enough, to a careful observer the determination of the sex of pupae, even of small Geometrid pupae like these, presents no great difficulty, and it was readily ascertained that all of the 46 pupae were males—a result entirely in agreement with the composition of ordinary broods reared as the outcome of a pairing between *dilutata* ♀ and *autumnata* ♂. This observation determines that, in the power of the male sex gene, there is no significant difference between any derived from the F_2 ♂♂ and those of pure *autumnata*.

The Pairing of filigrammaria ♀ and dilutata ♂.

Ova of this parentage were obtained with equal facility, but the number of salmon pink ones noted when the cage was opened only amounted to 19 out of 97. Clearly the pairing had been a success, but

from its meagre results in fertile eggs the physiological affinity between *dilutata* and *filigrammaria* must be exceedingly slight. Contrasting this with what is seen in the parallel *autumnata-dilutata* cross, where we obtain 100 % fertile ova, and with care an equal number of imagines, we see that the relationship between *filigrammaria* and *dilutata* is of a different order from that between *autumnata* and *dilutata*. From this it follows immediately that there is a definite physiological difference between *autumnata* and *filigrammaria* lifting the latter to a higher evolutionary level than the former—a fact which the perfect fertility of the two forms and their various crosses and back crosses could not have revealed save in the minor disturbance in the F_1 hybrid gametogenesis.

From the 19 fertile eggs five larvae hatched, and of these four died without feeding; the fifth struggled into its second instar and then followed the majority, their weakness thus confirming the greater divergence between *dilutata* and *filigrammaria* as compared with that between *dilutata* and *autumnata*.

The Pairing between dilutata ♀ and filigrammaria ♂.

This cross pairing gave absolutely no fertile ova in 1917; a repetition, however, in 1918 has provided me with a brood of which at least 20 % have changed colour and may possibly hatch during the present spring. As far as I have gone, the evidence of the present cross simply reinforces that of the last.

The Crossing of Cheimatobia boreata ♀ and O. autumnata ♂.

Owing to the obvious parallelism between the relationship of the genera *Cheimatobia* and *Oporabia* and that between *Nyssia* and *Lycia*, it occurred to me that hybridity between the first named generic pair might be feasible. Moreover, I considered that if this were so, further information might be gained as to the inheritance of apterousness in a group far removed from the well worked *Bistoninae*. In pursuit of these possible crosses I caged reciprocal pairs of *Cheimatobia boreata* and *Oporabia autumnata* together and awaited events.

In the cage containing *boreata* males and *autumnata* females not a single egg was laid; nor was the matter greatly improved in the other cage. Instead of tucking neat little cakes of ova in the folds of the muslin the *boreata* females scattered their eggs irregularly over the cage. This occurrence is generally to be regarded as a certain indication of failure in fertilisation, produced either by oviposition without copulation or by some mechanical hindrance to the passage of spermatozoa from the bursa copulatrix through the ductus bursae. In this

case the latter is the correct explanation for seven ova passed from green to pink only to fail to develop in spring.

Similar attempts to pair *O. autumnata* and *Cheimatobia brumata* proved utterly futile, no ova appearing at all.

IV. SUMMARY.

(1) The genus *Oporabia* is of Boreal origin and distribution; it comprises within its limits in its British range two species, *Oporabia autumnata* and *O. dilutata*, each possessing a well defined subspecies and numerous local races.

(2) All of these forms, no matter what their value, display enormous variability, and their variations occur in parallel series.

(3) All develop melanic and melanochoic races and aberrations.

(4) It is suggested that the subspecies *Oporabia filigrammaria* was evolved from *O. autumnata* on non-glaciated areas to the west of the British Islands of today; this event was caused during the Glacial period by the action, direct and indirect, of changed climatic conditions.

(5) The genesis of local races, it is further pointed out, is brought about by natural selection limiting the range of variation by the elimination of genetical strains less protected in any given habitat.

(6) Almost certainly, many of the changes exhibited, both those of racial and those of subspecific value, are true Lamarckian effects. In particular, food instincts in *O. filigrammaria*, period of emergence in the pinewood race of *O. autumnata*, are only explicable on such a basis.

(7) The differences in size between the various local races of *O. autumnata* are shown to be ontogenetic and to depend on the food plant.

(8) Such characters as mark both subspecies and local races, save that of size, are proved experimentally to be germinally fixed.

(9) Genetically distinct strains exist within the local races as was demonstrated by experiment.

(10) It is urged that the currently accepted explanations of melanism, in which the inducing cause is stated to be moisture followed by natural selection directed by the darkened resting places in manufacturing and rainswept areas, do not adequately account for the phenomenon, the premises for such an argument not having been correctly determined.

(11) To replace this theory it is suggested that changed metabolism favouring resistance to, or actually induced by, the use of food contaminated with metallic salts and other compounds affords a more comprehensive explanation.

(12) The influence of natural selection in establishing melanism, when once developed, is not excluded, although the results from prolonged and exhaustive work on *Polia chi* showed that no selection, favourable either to dark or to light forms, took place in the day time from 5.30 A.M. to 7.30 P.M.

(13) The inheritance of melanism in the genus *Oporabia* is not on Mendelian lines.

(14) The inheritance of the carpet band in the *latifasciata* form of *O. autumnata* is on a sex-linked basis exactly the same as that of the *lacticolor* aberration of *Abrazas grossulariata*.

(15) In all probability the inheritance observed in the crosses between *O. autumnata* and *O. dilutata* is likewise of the sex-linked type; it may, nevertheless, be influenced by mitotic anomalies.

(16) To the F_2 generation and in the back crosses the hybrids between *O. autumnata* and *O. filigrammaria* showed a perfect blend, no traces of Mendelian segregation being determinable.

(17) The evidence of the *autumnata-filigrammaria* crosses afforded no support to the multiple factor theory of size inheritance.

(18) In the F_3 generation a delayed or pseudo-segregation is manifested in which a portion of the brood still remain intermediate whilst the remainder appeared in a uniform pseudo-mutational guise. This strongly recalls the behaviour of *Oenothera Lamarckiana* and suggests that the phenomena displayed by that plant are those of hybridity and not of mutation.

(19) A female from the intermediate portion of the F_3 lot possessed three antennae.

(20) In the back crosses of the F_1 *filigrammaria* \times *autumnata* lots one back cross with *autumnata* sufficed to restore the *autumnata* condition; on the contrary, it required the operation to be repeated twice to bring the insect back to the *filigrammaria* facies.

(21) In the *autumnata-dilutata* crosses very great disturbances were observed: (1) the females in the cross between *O. autumnata* ♀ and *O. dilutata* ♂ emerged three months before the males and possessed no ovaries; (2) the reverse cross rarely produces females, never more

than one per brood; (3) when such are encountered they are indistinguishable from pure *O. autumnata*, are fertile, never (no matter how paired up) behave otherwise than as pure *autumnata*; (4) the *dilutata* ♀ × *autumnata* ♂ lot display a great lack of viability in the pupal condition.

(22) Both sets of males are fertile but no imago has ever been reared from back crosses in which they took part.

(23) A larva endowed with wings appeared in one of the *autumnata* ♀ × *dilutata* ♂ broods.

(24) Certain crosses including all three forms *autumnata*, *filigrammaria* and *dilutata* were obtained; owing to the influence of the *dilutata* female used in their production, the same failure in viability as marked the F_1 lot from *dilutata* ♀ and *autumnata* ♂ was manifested.

(25) The crosses between *O. dilutata* and *O. filigrammaria* failed, apparently through lack of physiological affinity.

(26) The haploid chromosome numbers of *dilutata*, *autumnata* and *filigrammaria* are 30, 38, 37 respectively.

(27) An almost perfect reduction division takes place in the gametogenesis of F_1 *filigrammaria* ♀ × *autumnata* ♂ hybrids, only one or two chromosomes failing to find mates.

(28) No pairing of homologous chromosomes takes place in preparation for the maturation divisions of either of the F_1 *dilutata* × *autumnata* hybrids; no reduction division, therefore, occurs.

(29) Bacterial disease in which even the germ cells were affected attacked the larvae in many cultures; in such broods gametogenesis was postponed, thereby necessitating delayed emergence. Though infected, females of the cross *autumnata* ♀ × *dilutata* ♂ appeared at their accustomed time.

(30) Fertile ova were obtained in the intergeneric cross between *Cheimatobia boreata* ♀ and *O. autumnata* ♂; all, however, failed to hatch.

(31) No ova were obtained from the reciprocal cross to this or from either of those possible between *Cheimatobia brumata* and *O. autumnata*.

NOTES ON THE INHERITANCE OF COLOUR AND MARKINGS IN PEDIGREE HEREFORD CATTLE.

By FRANCES PITT.

(With Plates VII—X.)

CONTENTS.

	PAGE
1. Introduction	281
2. The Typical Hereford	282
3. Variations Studied	282
I. Excessive White	283
II. Extension of Pigment	288
III. Pigment Round the Eyes	290
IV. Pigmented or "Dirty" Nose	294
V. Coat Colour	296
4. Some Notes on the History and Probable Origin of the White Face in the Hereford Breed	297
5. Summary and Conclusion	299

1. INTRODUCTION.

THE following notes and observations on the inheritance of colour and markings in pure-bred Hereford cattle are only published because, as far as the writer is aware, no work has been done on the genetics of this breed, and comparatively little on that of other kinds. These notes are necessarily imperfect and incomplete, for cattle, on account of their slow rate of increase, are bad subjects for the study of heredity, but such facts as have been noted and are here given may serve to draw the attention of other workers to a breed of which the genetics have hitherto been neglected.

The notes have chiefly been made on the pedigree Herefords belonging to Mr W. J. Pitt, of Bridgnorth, Shropshire. Nearly every beast bred for some years past has been photographed as a calf and the picture placed in a herd book under that of its dam; thus the produce of each cow can be seen at a glance. This system keeps a trustworthy record,

282 *Colour and Markings in Pedigree Hereford Cattle*

the collection of photographs enabling one to draw conclusions and arrive at results which would not otherwise have been apparent. Characters which appear to be fluctuating variations are shown by this book to be inherited as unit characters in a Mendelian manner. It must be mentioned that owing to the value of the material no experimental breeding has been attempted, though had it been possible to do so most interesting results might have been obtained. With cattle worth hundreds apiece¹ experimental breeding for undesirable points is out of the question in a herd run on commercial lines.

2. THE TYPICAL HEREFORD.

Before considering the manner in which different characters are inherited it will be necessary to give a description of the typical Hereford. It is a deep red beast, with white face and underparts, white feet, white at the end of the tail, and a white patch along the top of the neck. Sometimes there is a trace of red round the eyes. The stranger to the breed is generally impressed by the constancy of these markings; still, variations from the type do occur. It is hardly necessary to add that the Hereford is a very heavy fleshed beast, fattening rapidly, and is the premier beef breed of the world, having spread to every country where beef raising is a considerable industry.

Minor points that breeders attend to are: coat colour, which should be a rich purple red, not a yellow-brown; a clean, clear nose, without spots and markings; and the horns, which should be free from pigment at the tips.

3. VARIATIONS STUDIED.

I. *Reduction of the Pigmented area, or Excess of White*: This variation is the one most frequently met with, certain families being known to "throw white" occasionally. Pl. VII, fig. 2 shows a bad example of this departure from the type, in which the white markings have spread out and much reduced the coloured area. Pl. VII, fig. 3 shows another example, in which the animal has a streak of white down the spine and somewhat more than it should have in other parts. It will hereafter be shown that the variation is caused by a recessive factor that will be designated by the letter "W."

¹ In the autumn of 1918 a herd of 84 pedigree Herefords were dispersed at an average of over £500 each, including the stock bull, Ringer, which fetched 9000 guineas.

II. *Extension of Pigmented area, or "Dark Neck"*: Is a not uncommon departure from type. The white patch on the crest is lost, the pigmented area shows a general tendency to encroach on the white parts, the tail being often coloured to the end, and the limbs down to the ground. See Pl. VII, figs. 4 and 5, and grade + 3 on Pl. VIII. The character has been found to be produced by a dominant factor for which the letter "D" will be used.

III. *Pigment Surrounding the Eyes*: This is a ring of red round each eye, which varies in amount. As long as it is not excessive breeders do not object to it, and red, or "cherry" eyes are characteristic of some families of Herefords. See Pl. VII, figs. 5 and 6. The character is designated "R" in this paper.

IV. *Pigmented or "Dirty" Nose*: Spots of brown, and black, and of black and brown mixed, sometimes appear on the noses of Hereford cattle. Occasionally the whole nose is dark. Such "dirty" noses are greatly disliked by breeders, who invariably eliminate the bearers of them from their herds, yet dark noses continue to crop up in even the best-bred strains of cattle, and very often accompany the sought after and fashionable claret-coloured coat. "P" will herein be used for this character. See Pl. VII, fig. 7, and Pl. X, for examples of the "dirty" nose character.

V. *Coat Colour*: Two alternative characters are here dealt with, the first and dominant being the pale brown shade sometimes known to breeders as "yellow," and the deep plum tint called "claret" or "purple," which behaves as a recessive to the paler type of pigmentation. The lighter colour was at one time the more general, but having become unfashionable is not so common now. The pale brown factor is herein styled "B," and that for the "claret" coat is called "C."

I. *Excessive White.*

As already stated there are degrees of excessive white, which possibly indicates the presence of two or more complementary factors, such as give rise to the fluctuating character of "hooding" in rats, but the figures at present available are too small to permit of analysis. All that can be said with certainty is that too much white acts as a recessive to normal colouration. I first obtained evidence of this in the progeny of a bull called Lowland Paradigm (26986). He was an exceptionally fine animal, and his colour and markings were all that could be desired, yet five of his produce were badly marked, and more "white" individuals appeared in subsequent generations. The fact of the white appearing

284 *Colour and Markings in Pedigree Hereford Cattle*

in the first generation proves the genetic constitution of the parents. Both bull and cows must have carried the factor for too much white. The five normally marked cows with which Lowland Paradigm was mated, and which were proved by their produce to be heterozygous for the character in question, had by him seventeen calves, twelve being well marked and five badly. The ratio expected from the mating of heterozygotes is 3:1 when dominance is, as in this case, complete; but the difficulty of getting at the facts when working with such small figures is apparent, for the shifting of an individual from one class to another will make all the difference whether the result agrees with expectation or not. The figures obtained are 12:5, a really satisfactory agreement with expectation.

Lowland Paradigm was also the sire of thirteen calves from five cows believed to be homozygous for the typical markings. These calves were all normal in appearance. Half of them should according to theory have carried the factor for excessive white, but unfortunately the majority could not be tested as they were sold young, only three heifers being kept to breed from. These three young cows proved when bred to a bull known to carry the factor for white to be two pure dominants and one heterozygote. The latter, Olive Oil by name, has so far produced three well marked to one badly marked calf. This it will be noted is in exact agreement with the expected 3:1 ratio.

Lowland Paradigm's five badly marked calves from the cows that carried the factor for white were, as would be expected, pure for excessive white, i.e. they were extracted recessives. If we indicate those individuals homozygous for the typical markings by the letters NN , those bearing both factors by NW , then the calves referred to above will be of the genetic constitution WW ¹. That this assumption was correct was proved by their produce. Four out of the five were heifers and were kept for stock. Up to date these young cows have had 21 calves by heterozygous bulls, the result being 10 typically marked, to 11 with too much white. 11:10 is again a very fair approximation to the expected 1:1 ratio that results from the mating of a homozygous recessive with a heterozygote.

To take now the produce of another bull, by name Bounds Chance

¹ As the white face of the Hereford is dominant in crosses with distinct breeds, and even with species such as the bison, it seems that the white is due to the *presence* of a factor, and not merely due to the absence of that for normal pigmentation, so I have indicated excessive white by a capital W , and not by the small letter which is used to show the absence of a factor.

(29544). This was also a very fine typical animal, well marked, and most fashionably bred, yet evidently heterozygous for too much white. When mated with six cows of normal appearance but believed to carry the factor for white he sired 18 calves, of which 13 were well marked and 5 badly. Taking into consideration the smallness of the numbers, when the transposition of a unit will effect the agreement with expectation, this again is a fair approach to the 3 : 1 ratio. His matings with 13 cows homozygous for normal markings resulted in 39 typically marked calves in accordance with expectation. Theoretically half of these should be heterozygotes, but so far too few have been bred from to afford any evidence as to what proportion of them bear the factor for excessive white. Lastly Bounds Chance has had from four badly marked cows, presumably pure recessives, 16 offspring, 9 being too white, and 7 properly marked—expectation 8 : 8.

The produce of the above bulls may be set forth as follows:

		NN or NW		WW		
Lowland Paradigm	NW × 5 cows NW=	12	:	5		
Bounds Chance	NW × 6 cows NW=	13	:	5		
Totals ...		25	:	10		
Expectation ...		26.25	:	8.75		
		NN or NW		WW		
Lowland Paradigm	NW × 5 cows NN=	13	:	0		
Bounds Chance	NW × 13 cows NN=	39	:	0		
Totals ...		52	:	0		
Expectation ...		52	:	0		
		NN	NW	WW		
Bounds Chance	NW × 4 cows WW=	0	:	7	:	9
Expectation ...		0	:	8	:	8

Recessive Nature of the White Factor Illustrated by a Pedigree: Diagram 1 is a pedigree, traced through six generations, which illustrates in another manner the way in which excessive white crops up among normally marked cattle. The family shown has produced six individuals marked with too much white. The pedigree enables one to see at a glance, what has already been set out in the previous tables, that excessive white is due to a simple Mendelian factor acting as a recessive to that for normal colouration. The futility of the ordinary methods of elimination is also apparent. It must be added that the genetic constitution of the cattle shown in this pedigree is deduced from their entire produce, which of course are too many to be shown completely in the diagram.

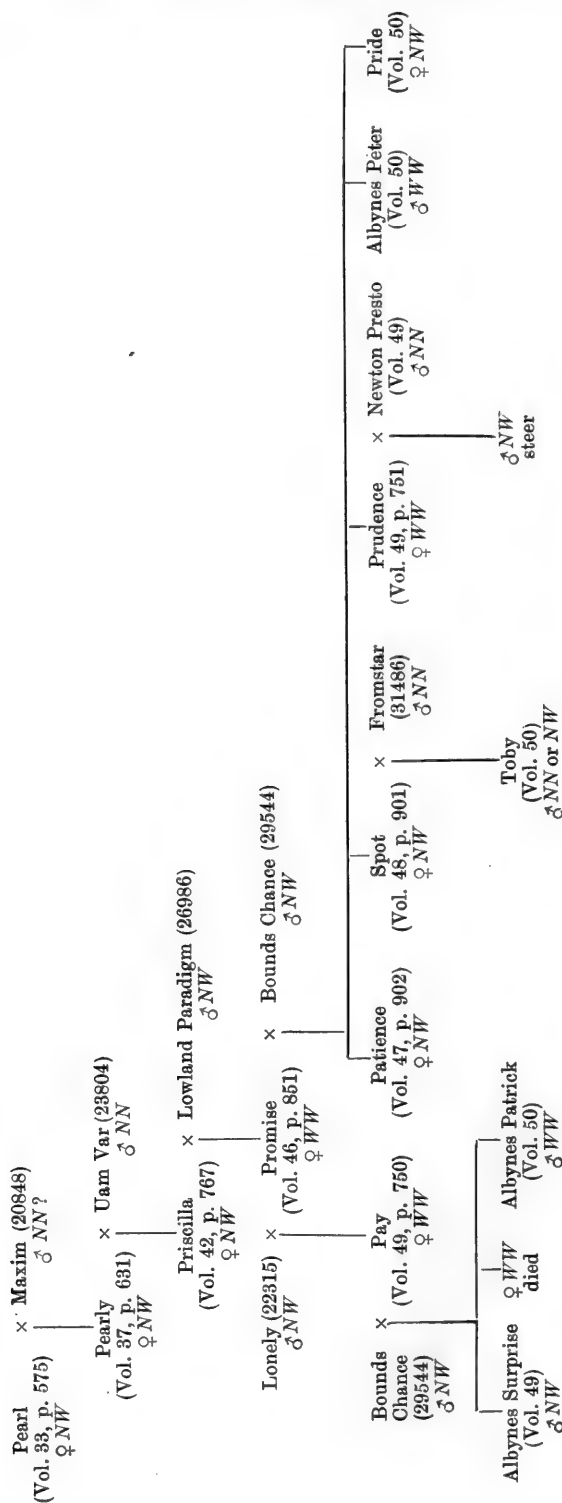


Diagram 1. Pedigree of a family which has produced six individuals marked with too much white and from which it will be seen that the factor for excessive white is a simple Mendelian recessive to that for normal colouration. The pure recessives in the above pedigree are indicated by the letters *WW*, the pure dominants by *NN*, and the heterozygous individuals by the letters *NW*.

Variability of the Somatic Expression of the Factor for White: Promise 2nd, the first pure recessive to appear in the above mentioned family, was not of the extremely badly marked type such as her daughter, Pay, shown on Pl. VII, fig. 3, but was a grade $-1\frac{1}{2}$ animal; however, her progeny have proved her pure for the white factor, and her first calf, as already mentioned, was an exceedingly white heifer. Her produce, by heterozygous bulls, have been 3 *WW*, and 4 *NW*, the three recessives being particularly badly marked. I have mentioned this to show that, though the somatic effect of the white factor varies somewhat, any beast having white beyond the shoulders may be confidently assumed to be a homozygote of the constitution *WW*. Of the four cows proved to be pure for white with which Bounds Chance (*NW*) was mated, two, Princess 2nd and Promise 2nd, were rather too white and two, Olivette and Pay, were exceedingly badly marked, yet genetically they behaved in a similar way. The latter had between them eight calves, four being normal, to four with too much white. The other cows had also eight calves, three with typical markings, and five badly marked. This shows, as stated before, that not only is the factor for excessive white variable in its somatic expression, but that individuals in which it does not reach its full expression are identical genetically with those in which it is fully displayed.

Summary: The above evidence shows that extra white in Hereford cattle acts as a simple Mendelian recessive to the normal type of markings, normal and badly marked cattle occurring in the ratio of 3 : 1 when heterozygous individuals are mated together. The factor for excessive white has been styled herein the *W* factor, the capital letter being used because the disappearance of pigment from the white areas is probably due to the presence of a special factor, and not merely to the absence of that for the typical markings. This conclusion is arrived at because the white face of the Hereford is dominant in crosses with most other breeds of cattle, and it is logically impossible to comprehend how a thing which does not exist can dominate that which is present. The dominance of the white face *must* be due to some special factor or gene, for the absence of factors for colour could hardly cause the disappearance of those for pigmentation from the gametes of fully pigmented breeds with which the Hereford happens to be crossed. If the white face and extremities are due to the presence of a special factor it becomes apparent that the extension of the white is almost certainly due to another similar factor, that which has been styled *W*. This can only show itself in the absence of the factor for the normal amount of pigmentation. The expression of the factor *W* varies somewhat, and

288 *Colour and Markings in Pedigree Hereford Cattle*

we have seen that some pure recessives are not so white as others, but so far all individuals bearing too much white that have been studied have given identical results when bred from.

II. *Extension of the Pigment or "Dark Neck."*

Pl. VII, figs. 4 and 5 and Plate VIII, grades + 2 and + 3 show the type of colouring referred to as "dark-necked"; this kind of pigmentation is apparently nearly but not quite completely dominant to the ordinary white-necked description of marking.

In the case of a cow called Shelsley Cypress, that was dark-necked, only two out of six calves, all by normally marked bulls, have shown any white on the neck. Presumably the cow is a pure dominant for the character, the sires being heterozygous, but dominance not being always complete¹ white shows in some of the heterozygous offspring. The following pedigree will make this clear. That one of

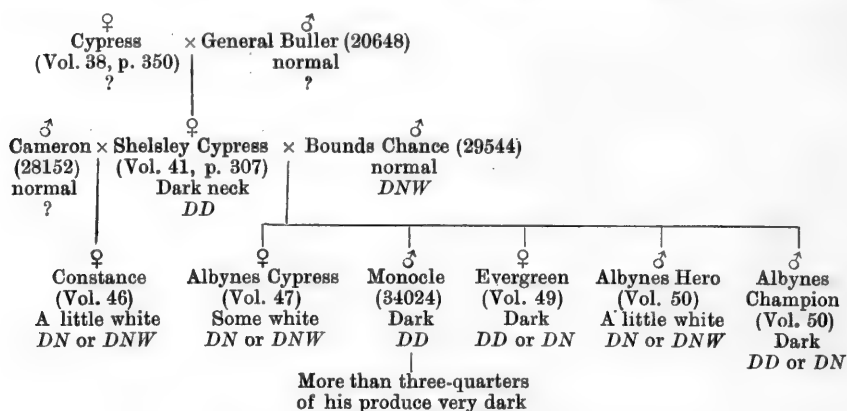


Diagram 2. Pedigree showing the Inheritance of the "Dark Neck" character.

D indicates the factor for the pigmentation of top of the neck, etc.

N indicates the factor for normal colouration, and

W is for excessive white.

the completely dark-necked calves was a pure dominant is shown by the fact that when subsequently used in a herd of non-pedigree general utility Herefords this young bull sired practically all dark calves. I have unfortunately not been able to obtain the exact figures relating to his produce, but his present owner, a very careful and accurate man,

¹ Further on in this paper it will be shown that the factor for excessive white has an inhibiting or neutralising effect on that for "dark neck," so that these calves may have been heterozygous for too much white.

assures me that, though all the cows were white-necked, in some cases very white, the proportion of white-necked calves has been exceedingly small. Again the evidence seems to point to the almost complete dominance of the factor for the extension of pigment.

Another case is that of a cow named Lady 4th, in which we have an individual apparently heterozygous for the character. She has two or three small spots of white hair on the shoulders. I have records of five of her calves. Judging by their markings these five offspring by normally marked bulls have been two dark-necked dominant homozygotes, one heterozygote with just a little white, and two well-marked homozygous recessives. Of course the heterozygotes are the class that one would have expected to be the most numerous, and possibly they are so in reality, for it will be shown presently that it is conceivable that sometimes the expression of the dark-necked factor is inhibited by the presence of another factor.

A third example is a cow called Playful, almost normally marked, but apparently carrying the factor for the extension of pigment. Three out of her five calves by well-marked bulls have shown no white whatever on the neck, while the two that had a little bore only very small patches.

This case and that of Lady seem to indicate that the sires with which they were mated likewise carried the factor for the dark neck. The bulls in question were known to be heterozygous for excessive white, and the suggestion is therefore made that the presence of the *W* factor inhibits the expression of that, which will be hereafter styled *D*, for dark neck. If this is correct it would explain the production of dark-necked calves by normally marked parents, and the complete and incomplete dominance of the dark neck in other cases. When only the factor for normal colouration is present *D* is a dominant, when that for *W* occurs it cannot fully express itself. How far this conjecture is in accordance with the truth only the collection of much more evidence will show. But the following case supports it—Gaiety, a perfectly marked cow, whose sire and dam were both well marked, had a calf by a bull called Wetmore Laurel, whose markings were typical in all respects, yet their calf was exceedingly dark, without a trace of white on the neck, and was an example of a grade + 3 animal. It was apparently homozygous for the extension of pigment, so each parent must have borne the factor for this character, the expression of which was inhibited by that for excessive white. This supposition is confirmed when we find that Gaiety's grand-dam was dark-necked. Unfortunately I have not been able to obtain any information concerning the appear-

290 *Colour and Markings in Pedigree Hereford Cattle*

ance of Wetmore Laurel's ancestors further than the first parental generation. The pedigree so far as the characters of the animals is known is set forth in the accompanying diagram.

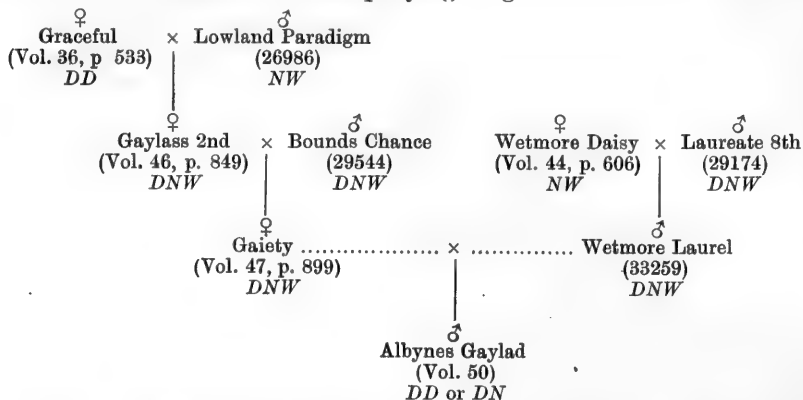


Diagram 3. A pedigree set forth to show how the dark-necked character, which is normally dominant, may be carried for several generations by typically marked cattle, its expression being inhibited by the factor for excessive white, until a suitable recombination of the factors occurs in which that for too much white is eliminated, and the dark-necked character shows again in full force.

The matings in which the "dark-necked" character has been concerned may be tabulated as follows:

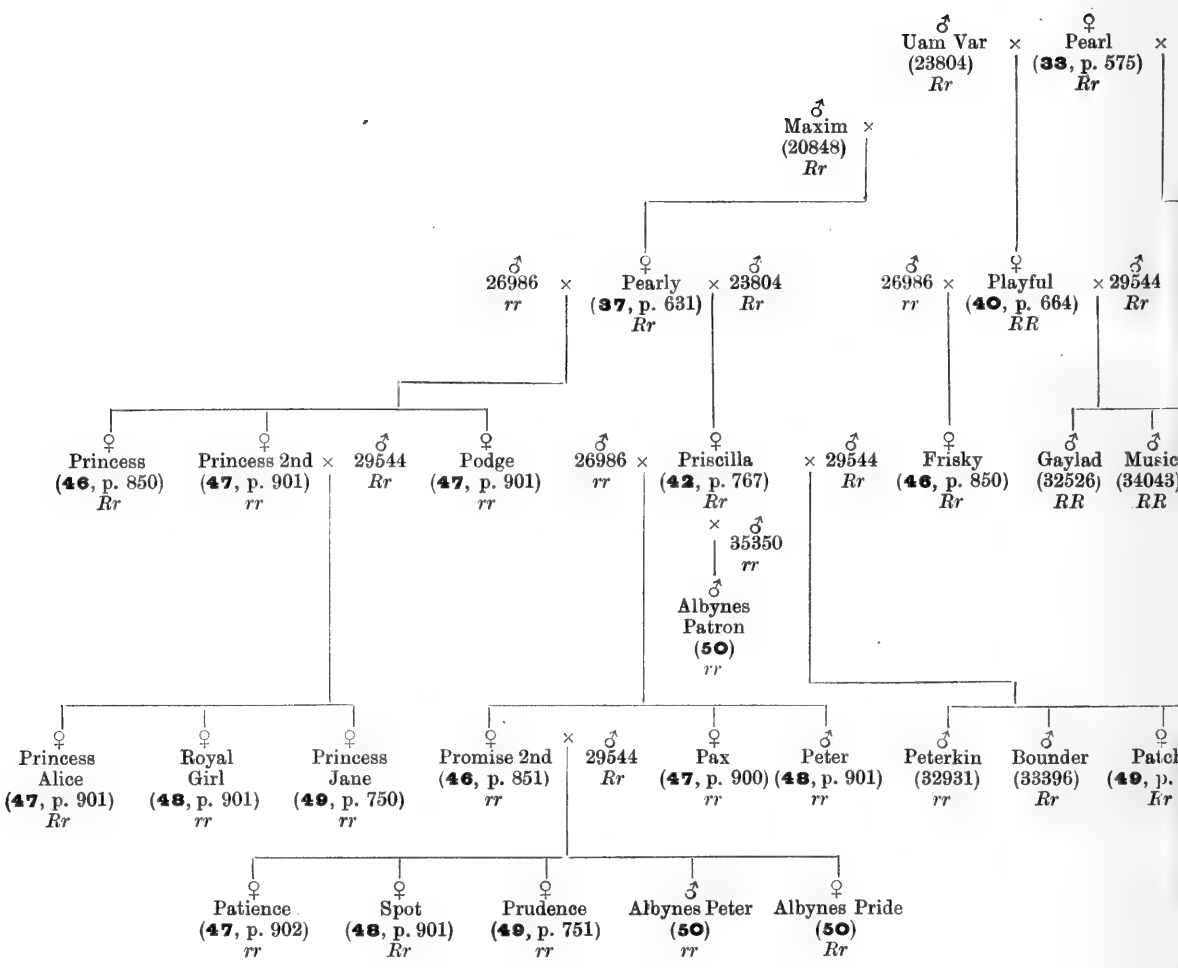
			<i>DD</i> or <i>DN</i>	<i>DN</i> or <i>DNW</i>	<i>NN</i> or <i>NW</i>
<i>DN</i> × <i>DNW</i>	{	Lady 4th × Newstead (30814) ...	1	0	1
		„ × Bounds Chance (29544) ...	1	0	2
		Playful × Lowland Paradigm (26986) ...	1	0	0
		„ × Bounds Chance (29544) ...	2	2	0
		Totals ...	5	2	3
<i>DD</i> × <i>DNW</i>		Shelsley Cypress × Bounds Chance (29544)	4	1	0
<i>DNW</i> × <i>DNW</i>		Gaiety × Wetmore Laurel (33259) ...	1	0	0

Summary: The evidence so far to hand leads one to believe that the "dark-necked" factor is dominant to that for normal colouration, from which it segregates in the ordinary Mendelian ratio, but it becomes more or less recessive in the presence of the factor for excessive white, which inhibits its full expression, so that a mating between outwardly normal individuals may give rise to a grade + 3 animal, as in the case of Gaiety and Wetmore Laurel just cited.

III. *Pigment Surrounding the Eyes.*

"Red-eyes," as the character is called by breeders, is quite common in English Herefords, about two-thirds of the cattle showing it (out of 110, 73 showed more or less colour about their eyes), though it has





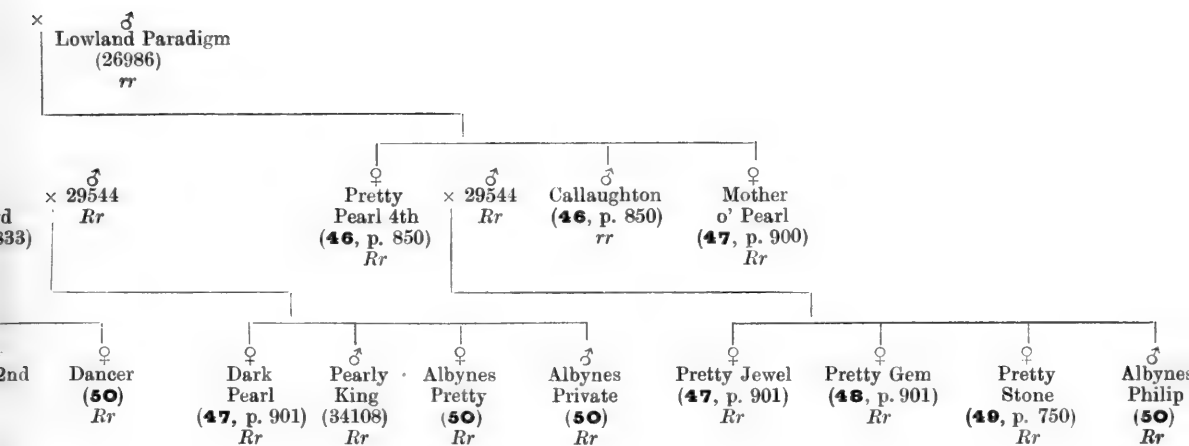


Diagram 4. Pedigree of a family of Herefords showing the manner in which pigment round the eyes is inherited.

Heavy pigmentation round the eyes is shown by the letters *RR*, the character being dominant, and the apparently the homozygous condition of it. Total absence of colour is shown by *rr*. The heterozygous condition, a small amount of pigment round both eyes, or only one eye coloured, is indicated by *Rr*.

Except in the first generation the sires used are indicated by their numbers only, which are as follows:

23804 Uam Var, slight red about the eyes, therefore *Rr*.

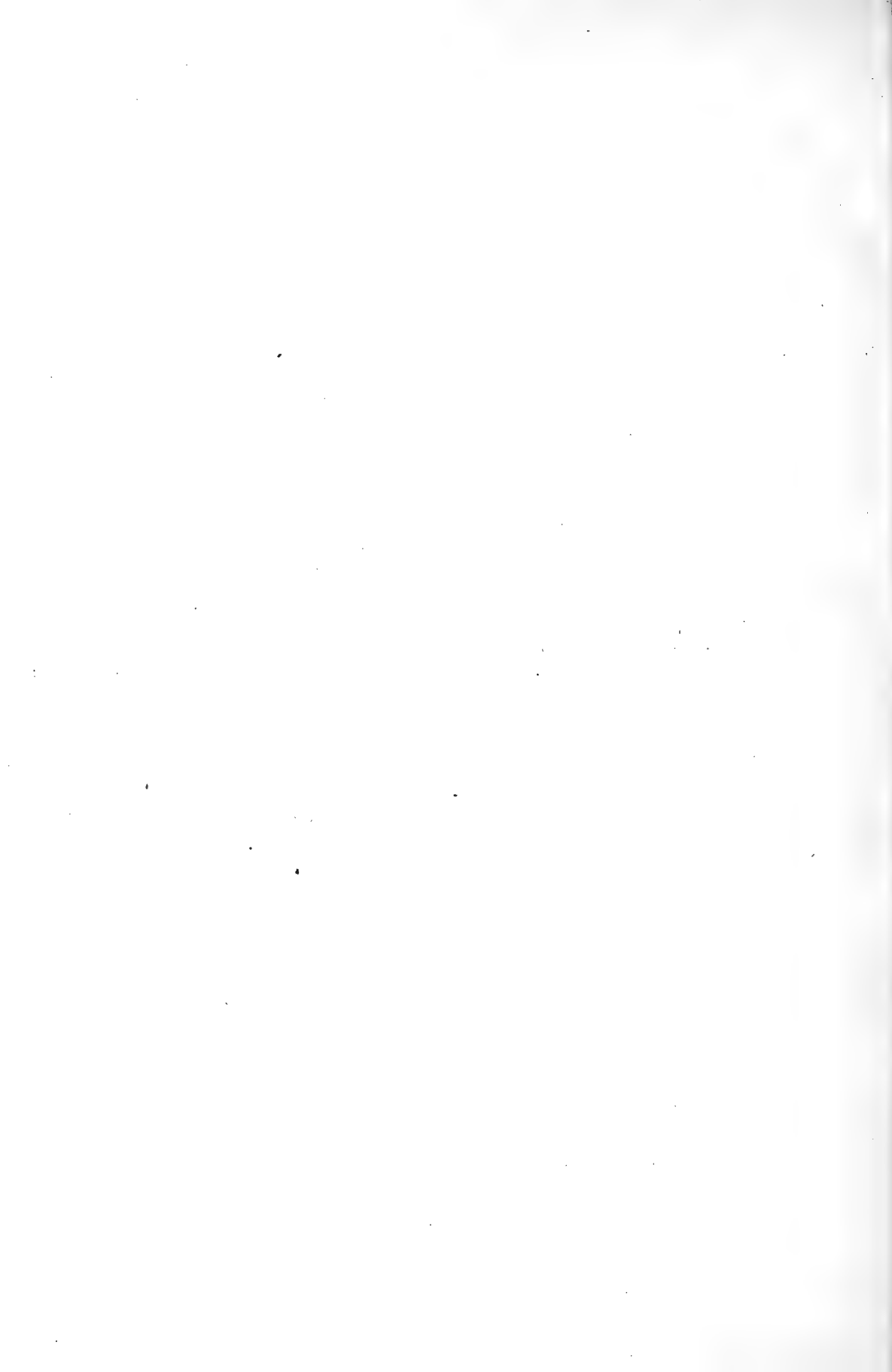
26986 Lowland Paradigm, no pigment round eyes, so indicated by *rr*.

29544 Bounds Chance, some colour in region of the eyes, *Rr*.

35350 Newton Glance, *rr*, having no pigment.

The figures in heavy type denote the volume of the Herd Book.

[To face p. 29]



never been the subject of selection, our breeders not regarding it as of any importance. In certain countries, Jamaica for one, cattle with pigment round the eyes are actually preferred on account of their supposed immunity to the attacks of flies and certain eye diseases. As far as English flies are concerned they certainly bite the eyes of red-eyed quite as badly as white-eyed Herefords, and I have been unable to see that the presence or absence of colour makes the least difference. However as some buyers for export choose red-eyed cattle the character has after all a certain interest for the breeder.

The coloured area varies from a comparatively large circle of red round each eye (Pl. VII, figs. 5 and 6) to the merest trace of pigment on one eye (Pl. IX, *F*). It is not uncommon for one eye to be well marked and the other plain (Pl. IX, *C*). When this is so, and in cases where only a little pigment is present round each eye, we have apparently expressions of the heterozygous condition.

The accompanying diagrams (4 and 5) show that the presence of pigment round the eyes is dominant to its absence, segregation following the simple Mendelian ratio. But dominance is not so complete as, for instance, in the classical case of the round and the wrinkled pea, and, as mentioned above, heterozygotes generally betray their constitution by the reduced amount of pigment present.

The character appears to segregate independently of other pigmentation factors, for I have seen very white cattle with red eyes, and very dark ones with white eyes; but it is possible that the red-eyed factor can only reach its full somatic expression when in company with that for extension of pigment, and that the factor for normal colouration inhibits its full development. This is suggested because specially heavy pigmentation round the eyes has been chiefly met with in company with absence of white from the neck. Out of 18 dark-necked cattle 14 had red eyes, of which 3 had conspicuous pigmented rings round their eyes, *but* one very red-eyed calf had a fair amount of white on the neck. The examination of the 110 cattle already referred to gave the following figures:

	56 normally marked Herefords with red eyes
	14 dark-necked with red eyes
	3 very white with red eyes
Total ...	73 red-eyed cattle
	28 normally marked white-eyed cattle
	4 dark-necked white-eyed ones
	5 excessively white and with white eyes
Total ...	37 white-eyed Herefords

292 *Colour and Markings in Pedigree Hereford Cattle*

Though the records of a number of matings show that the presence of pigment round the eyes is dominant to its absence, the evidence is insufficient to show to what extent, if any, the factors affect each other. It is certain that the heterozygotes tend to exhibit less pigment than the pure dominants, but the accurate division of one from the other by appearance alone is a somewhat difficult matter, so it is probable that some individuals classed as *Rr* are really *RR* (the "red-eyed" character is herein styled *RR*), and as I have already mentioned it is possible that the presence of the factor for normal markings tends to inhibit the full expression of that for "red-eyes"; only breeding will reveal the genetic constitution of these doubtful animals, but as, at the time of writing, the majority are calves or yearlings it will be some years before any certain conclusion can be arrived at.

The results achieved so far may be summarised as follows: 18 cows, believed *Rr*, had by 5 *Rr* bulls, 54 calves, 42 red-eyed to 12 white-eyed recessives. 42 : 12 is a fair approximation to the 3 : 1 ratio which in this case gives an expectation of 40·5 : 13·5.

As already explained too few of the red-eyed class have been tested by breeding for their genetic constitution to be anything but guess work. Some have hardly any pigment round the eyes, others a considerable amount, the colouring being so heavy in the case of seven as to make one convinced that they at any rate are homozygous for the character, which gives a result of 7 *RR* : 35 *Rr* : 12 *rr*. This makes the *RR* class too small, expectation being 13·5 : 27 : 13·5.

Matings of heterozygotes with recessives give the following results: 7 cows of the constitution *Rr* had by 5 *rr* bulls 17 calves, 8 red-eyed to 9 white-eyed. Three *rr* cows when mated with an *Rr* bull had 10 calves, being 4 *Rr* to 6 *rr*. Total 12 *Rr* : 15 *rr*; expectation being 13·5 : 13·5.

Considering the smallness of the figures dealt with the results support the general conclusions.

With regard to *Rr* × *RR* matings the evidence is unfortunately still more scanty, but what there is agrees with theoretical anticipations.

A *RR* cow had four calves by an *Rr* bull, two of the produce being undoubtedly pure dominants, and the other two heterozygous for the character. This cow also had a calf by a white-eyed bull which was clearly of the constitution *Rr*.

Summary: It is evident from the foregoing evidence that the presence of pigment round the eyes of Hereford cattle is dependent on a single dominant factor, which is allelomorphic to its absence, and that this

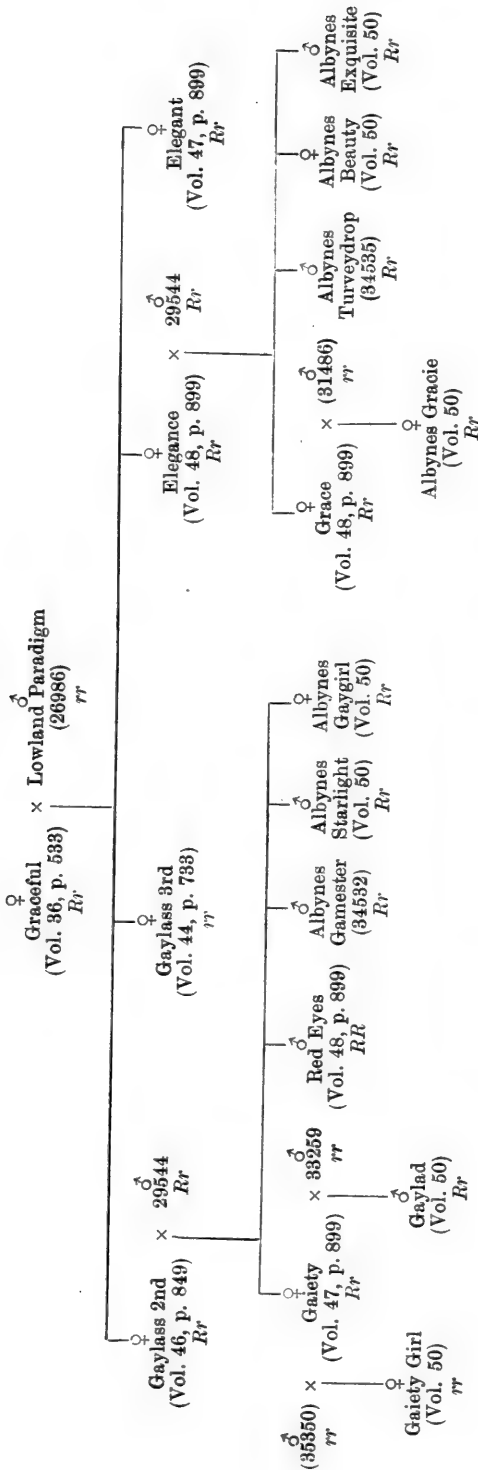


Diagram 5. Pedigree of a family of "Red-eyed" Herefords.

RR indicates heavy pigmentation round the eyes.

Rr is for less colour, in some cases only one eye being red—presumed to be the heterozygous individuals.

rr indicates absence of pigment; i.e. the pure recessives.

The sires used are to save space shown, with one exception, by their numbers. Their names are as follows :

29544—Bounds Chance.

33259—Wetmore Laurel.

35350—Newton Glance.

31486—Fromestar.

factor segregates, independently of other pigmentation factors, in the simple ratio of 3:1, or really 1:2:1. Dominance is not in all cases quite complete, so that the *RR* and *Rr* classes are not always easy to distinguish with certainty. It is possible and even probable that the factor for normal colouration has a modifying, or partially inhibiting effect on the full somatic expression of that for "red eyes," so that a normally marked red-eyed beast would not have such well-marked eyes as a dark-necked red-eyed one, though both would transmit the red-eyed factor in equal purity to their offspring.

IV. *Pigmented Nose.*

Pigment sometimes appears on the nose in Hereford cattle in quantities varying from a mere spot or two up to a completely dark nose. All forms of dirty nose are objected to by breeders, but brown spots alone are not considered so bad as black, or "blue" (dilute black). Very often an examination of a dark nose will show that both pigments are present, this being perhaps a commoner type of dirty nose than either pure black or brown. My observations concerning the inheritance of brown pigment on the nose are insufficient to justify any conclusions being formed. More information has been collected concerning black, and black and brown combined. The first point that appears is that this form of dirty nose is usually found accompanying that deep rich coat colour known as claret. Out of 41 Herefords examined, which were of this tint, 15 had pigment on the nose, and 26 had clean noses. But pigment on the nose *can* occur independently of the "claret" coat, as I have met with two pale brown, or yellow-brown animals that had well spotted noses. These were a cow called Olive Oil (see Pl. VII, fig. 7) and her bull calf Oliphant. As this cow was without a trace of the fashionable plum tint, and the marks on her nose were particularly black, it shows that the pigmented nose is not necessarily correlated with a claret coat, and the factors for the two characters can segregate independently, yet there appears to be some association between them, as out of 11 "yellow" coated animals examined the two mentioned above were the only ones showing any trace of colour or marking, the rest having perfectly clean noses, though in the case of claret-coloured cattle the proportion rises to more than half.

I have mentioned that black and brown pigments are often combined in the dirty nose, and I have notes concerning the inheritance of such a type of nose through a pedigree of three generations. This pedigree is fully set out in the accompanying diagram, and from it, it will be seen

that not only does the presence of pigment on the nose behave as a unit character, but that it acts as a simple Mendelian dominant to the absence of the pigment; expectation being exactly realised in every instance.

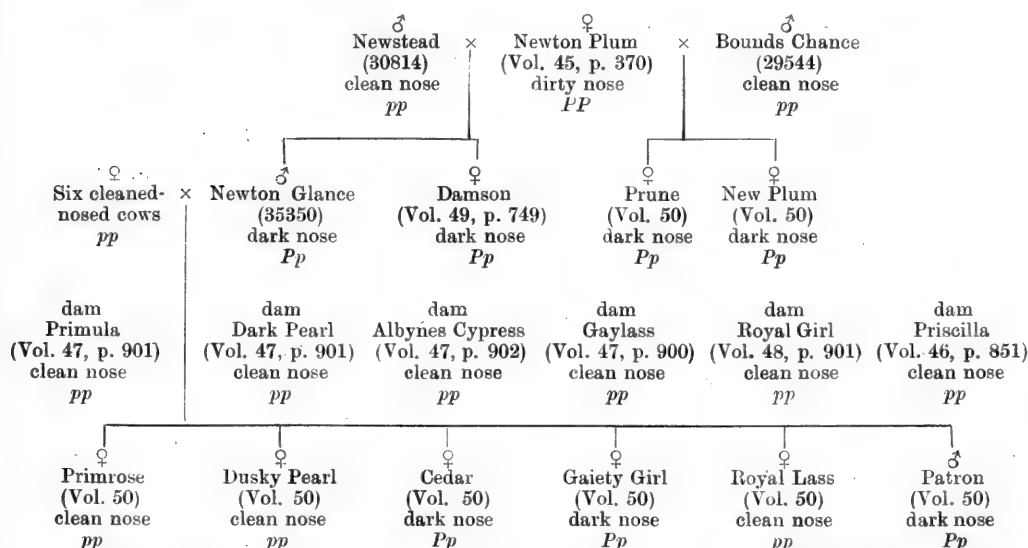


Diagram 6. Pedigree showing the inheritance of the dark or "dirty" nose through three generations. The factor for pigment on the nose is indicated by *P*, and its absence by *p*.

Except for the "purple" coat the dirty nose character is inherited independently of other factors. I have seen a very white cow (grade - 3) with a heavily spotted nose, and dark cattle (grade + 3) with perfectly clean noses. The figures collected in relation to this are as follows:

	Clean nose	Spotted	Heavily pigmented
Dark-necked + 3 and + 2 ...	7	1	0
Very white - 2 to - 4 ...	7	0	1
Claret coloured ...	26	0	15
Pale brown ...	9	0	2

The matings of clean-nosed with dirty-nosed cattle that I have records of may be summarised as below:

	<i>PP</i>	<i>Pp</i>	<i>pp</i>
<i>PP</i> × <i>pp</i> Newton Plum × Newstead (30814) ...	0	2	0
do × Bounds Chance (29544) ...	0	2	0
Total ...	0	4	0
Expectation ...	0	4	0
<i>PP</i> × <i>pp</i> Six clean-nosed cows × Newton Glance (35350) ...	0	3	3
Expectation ...	0	3	3

296 *Colour and Markings in Pedigree Hereford Cattle*

Summary: The dirty nose is clearly a unit character, dominant to the clean nose, and segregating independently of the other characters with the possible exception of the factor for the "purple" coat. As 15 out of 41 claret-coated cattle showed a spot or two of pigment on the nose some connection seems certain, but as the dark nose appears occasionally in company with the pale brown or "yellow" coat it is evident that the association is not complete.

V. Coat Colour, with reference to the Claret and Pale Brown shades.

The rich "claret" or "purple" colour is a dark plum tint which of late years has found much favour with breeders, though at one time a pale brown or "yellow" coat was preferred. In hot climates the dark coat is particularly sought after as it does not become so faded and bleached as the paler red-browns. "Claret" is the deepest shade found in Hereford cattle, "yellow" (actually a pale red-brown) being the lightest, but intermediate shades are often met with. Not only are there shades between the two extremes but the cattle vary much according to the time of year and the state of their coats, so that it is often most difficult, if not impossible, to be sure how a beast should be classed. For this reason many animals have had to be excluded from the following tables and calculations, in which only those that clearly belong to one class or the other have been included.

The claret factor proves to be recessive to that for pale brown, as the matings of seven "yellow" Hereford cows with two purple-coated bulls resulted in 14 pale brown calves to one dark one. This is somewhat surprising, as, judging by analogy with chestnut and bay horses, one would have expected the paler colour to prove the recessive. That the purples are really pure recessives is proved by the produce of purple \times purple matings, which with one exception were all dark. The exception was the calf of a doubtful cow, which was probably a heterozygote and should not have been included among the pure purples.

The results may be set forth as follows, the pale brown factor being shown by the letter *B*, and that for claret by *C*:

7 matings $BB \times CC = 14 BC : 1 CC?$	<i>Expectation 15 BC.</i>
10 matings $CC \times CC = 33 CC : 1 BC?$	<i>Expectation 34 CC.</i>

I have no records of the results of $BC \times BC$ and $BC \times CC$, and it can be only by keeping most careful notes over a number of years that the heterozygous matings will be worked out.

The pale brown coat factor is clearly inherited independently of all other pigment characters, but that for claret is rather frequently accom-

panied by those for red eyes and "dirty" nose. 52 beasts gave the following figures:

Excessive White.	
Pale Brown 11	Claret 41
1 <i>WW</i> : 10 <i>NN</i> or <i>NW</i>	2 <i>WW</i> : 39 <i>NN</i> or <i>NW</i>
Dark Neck.	
Pale Brown 11	Claret 41
3 <i>DD</i> or <i>DN</i> : 8 <i>NN</i>	8 <i>DD</i> or <i>DN</i> : 33 <i>NN</i>
"Dirty" Nose.	
Pale Brown 11	Claret 41
2 <i>PP</i> or <i>Pp</i> : 9 <i>pp</i>	15 <i>PP</i> or <i>Pp</i> : 26 <i>pp</i>
Red Eyes.	
Pale Brown 11	Claret 41
7 <i>RR</i> or <i>Rr</i> : 4 <i>rr</i>	37 <i>RR</i> or <i>Rr</i> : 4 <i>rr</i>

Summary: Coat colour in Hereford cattle is controlled by two pigment factors, "*B*" for the pale brown coat, which is dominant over "*C*," the darkest shade, which is a deep rich purple or claret. Intermediate tints are probably attributable to the heterozygous condition, but further evidence is wanted on this point. The factors for coat colour segregate independently of those controlling the distribution of pigment on the body, but there appears to be some association between the *C* factor and those for red eyes and the "dirty" nose. It is undoubtedly significant that more than a third of the dark-coated beasts should have pigmented noses, while the proportion in the pale brown cattle is 1 : 4.50.

4. NOTES ON THE HISTORY OF HEREFORD CATTLE WITH REFERENCE TO THEIR MARKINGS AND VARIATIONS.

Writing in 1627 Speed said that no "place in England yieldeth more or better conditioned cattle than Herefordshire" (1). In 1788 we find the colour of the animals was "a middle red with a bald face" (2) showing that the type as now established was even then common. By 1804 "the prevailing colour" was "a reddish brown with white face" (3). Other types prevalent at that time were "whites"—really pale

roans,—“greys” or deep roans, entirely red cattle, and many with blue mottled and ticked faces (10). Many of the “red with white face” variety were much whiter than we should like in these days, the white extending right along their backs, and being splashed upon sides and flanks. Some of these cattle had large patches of red round the eyes and across the face. The four principal types are illustrated by pictures of four celebrated bulls in the first volume of Eyton’s Herd Book (pub. 1846). The statement that the majority of the Herefordshire cattle belonged to the “red with white” face variety is borne out by an examination of the first two vols. of the herd book, which contain the pedigrees of 551 bulls, whose markings are also recorded in 326 cases. They were 191 “red with white face,” 98 “mottle-face,” 30 “grey,” and 7 “light grey.” The red with white face class had a majority of 56 over all other types combined. Twelve years later, out of 235 bulls (registered in Part 1, vol. II of the Herd Book, 1853) only two were recorded as “greys,” the rest being “red with white face.” By 1877 the breed was so uniform in appearance that the cattle were no longer described when registered in the herd book.

All this goes to show that a heavy fleshed breed of cattle (of which the oxen fattened readily when their days at the yoke were finished) was found in Herefordshire early in the 17th century, and that by the middle of the 18th century the conspicuous “red with white face” type was well-established, though the varieties, grey, mottle-face, etc., were equally accepted as good Herefords, but, fashion setting in favour of the red, these varieties were ultimately weeded out, so that the word Hereford came to signify a red beast with a white face. But the interesting question is how did the well-known white face arise, for it is not only such a marked character but is nearly always dominant even in crosses with distinct species like the bison (12, p. 533). Early writers refer to whole red cattle like the Devons (5 and 8), and the appearance of the white face is variously attributed to the introduction from Flanders of white-faced cattle towards the latter part of the 17th century (10, p. 11), to crosses with the old white Welsh cattle (10, p. 19), to the use of white-marked bulls from the north (10, p. 27), and to the sudden appearance of a white-faced calf in a herd of dark cattle. This case rests upon the authority of the Mr Tully who was a famous breeder in the early part of the 19th century. He says “About the middle of the last century, the cowman came to the house announcing as a remarkable fact that the favourite cow had produced a white-faced bull-calf. This had never been known to have occurred before; and as a curiosity it

was agreed that the animal should be kept and reared as a future sire" and "the progeny of this very bull became celebrated for white faces" (8).

This calf, born about 1750, appears to have been a true sport or mutation, and as he had considerable influence on the breed it is possible that we here have the origin of the strongly dominant white face as it at present exists, but it must not be forgotten that we find it recorded that by 1788 the white-faced variety was so widely spread as to be considered typical of the cattle of the county, so probably there were many white-faced beasts in existence prior to 1750, for we can hardly credit that the progeny of one bull would have over-run the district in the space of 38 years.

We see from these few notes that the Hereford has arisen by selection from the nondescript cattle bred in the county of Herefordshire during the 17th and early part of the 18th centuries, that all sorts of colours and markings prevailed, among which the red with white face was most common, and that it is probable a mutation occurring in a herd of dark cattle helped to fix this characteristic, which, when the beef qualities and other points of the breed began to receive attention, became the most popular type of marking among breeders, other colours being eventually eliminated, so that it is only in a few small variations and departures from type that we see in the modern Hereford any survival of the variously marked and coloured cattle of the early days of the breed.

5. GENERAL SUMMARY AND CONCLUSION.

The evidence that has been gone into in these pages establishes the fact that Mendelian inheritance is the rule with regard to the colours and markings of Hereford cattle, each character being controlled by a separately heritable factor, so that it would be possible, were experiments with such slow breeding and valuable animals a financial possibility, to combine the factors for all the characters mentioned in a single beast.

We have seen that excessive white is a simple recessive to the typical form of pigmentation, that extension of pigment is due to a dominant factor, which is neutralised in its action when it chanches to be combined with the *W* factor, so that a normally marked beast might carry both characters, and transmit them separately, or combined, to its progeny. If this animal had a pale brown coat, and was heterozygous for the recessive purple-coat factor, and had in addition a dirty nose and red eyes, we should have such a beast as that referred to above, combining

300 *Colour and Markings in Pedigree Hereford Cattle*

in one individual all the characters that have been dealt with in this paper.

Lest it should be thought that normal markings are always produced by the combination of the *W* and *D* factors I may say there is undoubtedly a special factor for the typical form of pigmentation, as many strains of Herefords breed pure to it and never throw either dark or white variations. Were it otherwise, any and every line would be liable to produce individuals both too white and too dark, which is not the case.

Though the characters are inherited as clear and separate entities there is some indication of association between purple (or claret) coat colour and the dark nose, but this is certainly not complete, as badly spotted noses have been met with on pale brown cattle.

There are undoubtedly many other characters than those which have been more or less dealt with which would repay study, some of considerable economic importance, as, for instance, the ability to put on the maximum amount of flesh on the minimum amount of food. There is some slight evidence that dark-coated cattle do not feed so quickly as pale brown but put on their flesh more evenly, and that the "yellow" feed faster, getting excessively fat¹, but tend to become patchy and uneven, the fat accumulating in lumps. As the evidence on these points is not sufficient to be conclusive they are only mentioned here to show that the study of heredity in this breed may have considerable importance for the practical as well as the scientific man.

The notes on the history of the Hereford breed show that the pigimentary characters are survivals from earlier times when no selection was practised of colour or markings, and that the faults which appear and reappear in the present day Herefords are not, as some breeders believe, spontaneous variations, but due to Mendelian factors, carried by apparently normal animals, which cause the characters concerned to appear when a suitable combination of gametes gives them the opportunity.

There can be no doubt that the modern Hereford has been evolved from the old cattle found in the County of Hereford by a purifying process, by selecting and selecting from what was originally most heterogeneous material, until the more or less homogeneous breed as we know it to-day has resulted.

That many if not most of our domestic breeds have been produced in a similar manner can hardly be doubted. It is by selecting and re-

¹ It is stated (11, p. 120) that yellow mice are prone to get exceptionally fat.

arranging their characters that the breeder has produced his great results, *but* has this artificial process any likeness or parallel to the natural evolution of a species—can we visualize the production by any such process of a geographical race of a mammal, bird, or insect?

EXPLANATION OF PLATES VII—X.

PLATE VII.

- Fig. 1. *A Perfectly Marked Hereford*, Grade 0. A prize winning show heifer, Albynes Beauty, at fifteen months old. Note the patch of white on the neck, the white face, under-parts, brisket, and end of the tail, that the feet are white, and that her nose is clear and unspotted, also that her horns are free from pigment at the tips. In colour she is a rich red.
- Fig. 2. *Excessive White*. Olivette, a grade -3 cow.
- Fig. 3. *Excessive White*. Pay, a grade -4 animal.
- Fig. 4. "Dark Neck," or *Extension of Pigment*. Shelsley Cypress, a very dark grade +3 cow.
- Fig. 5. *Extension of Pigment*, also "Red Eyes." Graceful, a dark grade +3 cow with red eyes.
- Fig. 6. "Red Eyes." Belladonna, a red-eyed cow, and a good example of this character.
- Fig. 7. "Dirty" Nose. Olive Oil, a cow with a black spotted nose, but a pale brown coat.

PLATE VIII.

Grades of pigmentation in Hereford Cattle, 0 being the normal.

PLATE IX.

Variations of the "Red-Eyed" Character in Hereford Cattle.

PLATE X.

Pigmentation of the nose in Hereford Cattle, "a" being a completely dark nose, and "d" a clean unspotted one. The extreme "a" is rare, but "b" and "c" are not uncommon.

LITERATURE CITED.

1. SPEED, 1627. *England, Wales, and Scotland Described*.
2. MARSHALL, 1788. *Rural Economy of Gloucestershire*.
3. DUNCUMB, 1804. *History and Antiquities of the County of Hereford*.
4. PARKINSON, 1810. *Breeding and Management of Live Stock*.
5. YOUATT, 1835. *Cattle, Their Breeds, etc.*
6. EYTON, 1846. *Hereford Herd Book*, Vol. I.
7. ——— 1853. *Hereford Herd Book*, Vol. II. (Reprinted, 1862.)

302 *Colour and Markings in Pedigree Hereford Cattle*

8. ROWLANDSON, 1853. "Farming in Herefordshire." *Journal R. A. S. E.*, Vol. XIV.
9. DUCKHAM, 1863. Lecture at the Royal Agricultural College, Cirencester.
10. MACDONALD and SINCLAIR, 1886. *History of Hereford Cattle.*
11. BATESON, 1909. *Mendel's Principles of Heredity.*
12. BABCOCK and CLAUSEN, 1918. *Genetics in Relation to Agriculture.*

(Note: the numbers after the bulls' names, and the volume and page numbers after the cows' names, given in the pedigrees, etc., refer to the Herd Book of Hereford Cattle, and will enable any one to trace the full ancestry of the animals in question.)



Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



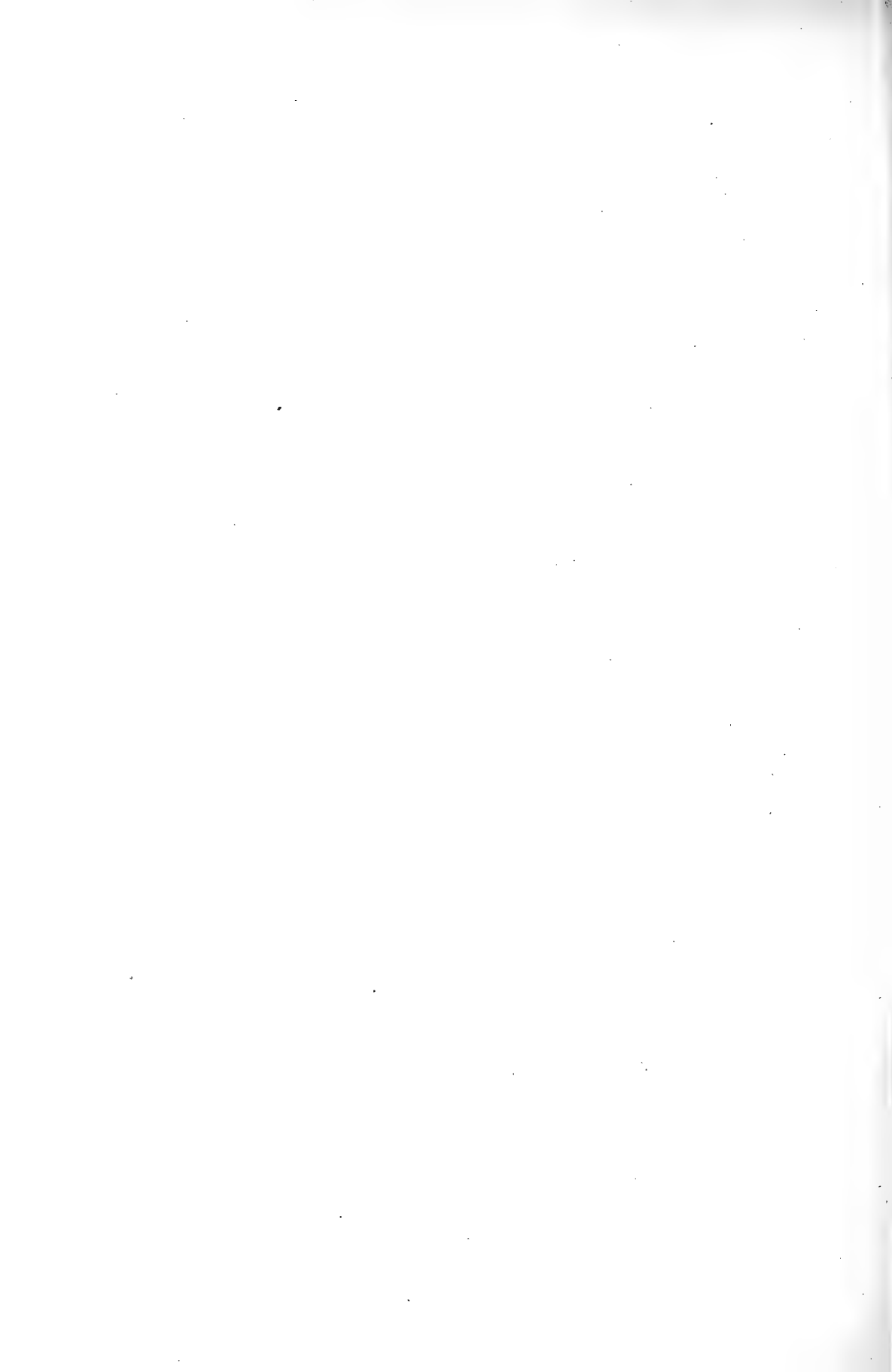
Fig. 5.

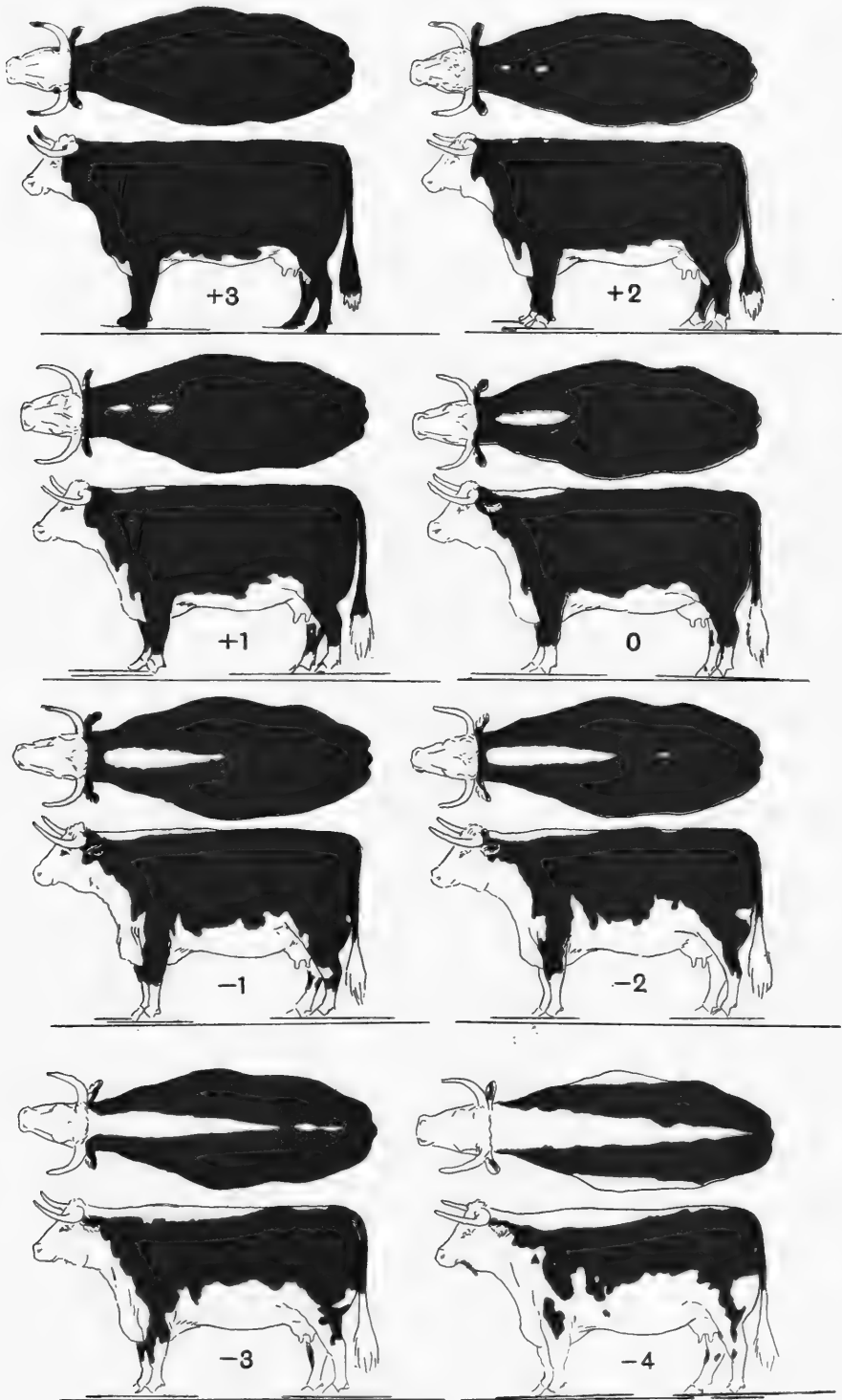


Fig. 6.

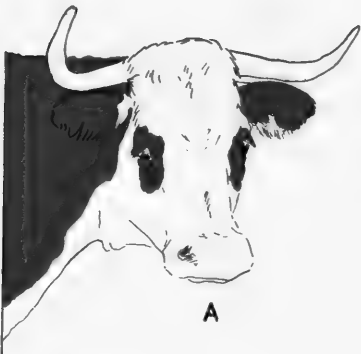


Fig. 7.

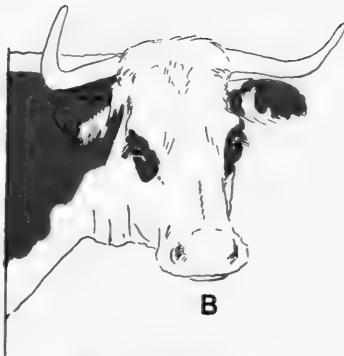




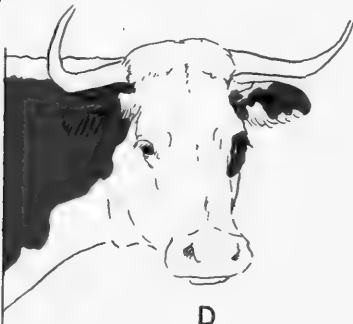




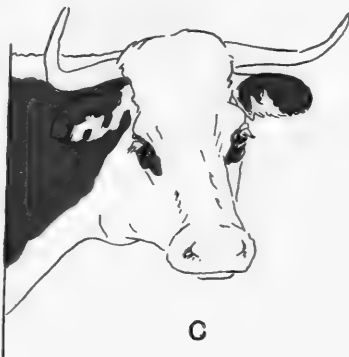
A



B



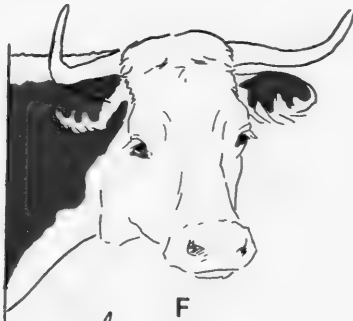
D



C



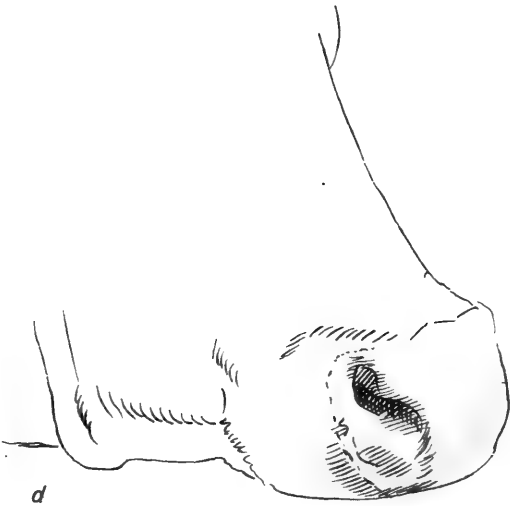
E



F



G



THE GENETICS OF THE DUTCH RABBIT— A CRITICISM.

BY R. C. PUNNETT.

(With two Text-figures and Plate XI.)

IN the earlier days of Mendelian studies attention was naturally focussed upon characters which were clean cut, and offered simple material with which to test the validity of the principle of segregation. But even in those days there was plenty of evidence for the existence of cases in which a cross between two true-breeding strains led to the production of a series of forms in F_2 , ranging between the two extremes characteristic of the parental types. Under peculiarly favourable circumstances it was found possible to frame an interpretation on strictly factorial lines, involving but a small number of factors. The pigmentation of the Silky fowl¹ was a case in point, analysis shewing that the apparently continuous range from the unpigmented to the fully pigmented condition could be interpreted in terms of three factors only, viz. (1) a factor for pigmentation, (2) a factor for pigment inhibition, and (3) the sex factor, which in the female operates to produce a somewhat higher grade of pigmentation than in the male of corresponding genetical composition. Owing to the diverse nature of the three factors the analysis of a continuous series was here much simplified, since specific tests of the factorial interpretation could be readily devised and carried out. Many cases, however, present greater difficulties to analysis, because we have no grounds for supposing that there is a qualitative distinction between the various postulated factors. For example, in dealing with the inheritance of size or weight, we may encounter cases where the F_2 generation is composed of a series passing imperceptibly from the one extreme to the other. As a typical instance may be cited that of the *Hamburgh* × *Sebright* cross in poultry, of which an account was given in the earlier pages of this *Journal*². It is possible, as was there pointed out, to bring such cases into line with the

¹ Bateson, W., and Punnett, R. C., *Journ. Genet.* Vol. i, 1911.

² Punnett, R. C., and Bailey, P. G., *Journ. Genet.* Vol. iv, 1914.

factorial hypothesis if we assume the existence of several factors each behaving in a similar way. But in dealing with such characters as those involving size and weight, which are so susceptible to alterations in the environment, the difficulties in the way of complete analysis are practically insuperable, and the interpretation in factorial terms must rest largely upon analogy.

The difficulties are not, however, so great in another group of cases where we encounter these apparently continuous series, viz. the group of pattern cases in animals, where the coat may range from almost white up to self-colour. As compared with size and weight they offer the great advantage that the pattern is practically constant throughout life, and apparently independent of extraneous influences such as food and warmth. Records can be obtained without the necessity of rearing all animals to maturity. The best known and most fully investigated example of a continuous series among pattern cases is that of Castle's rats, where the evidence shews that animals exhibiting the recessive hooded character may range from almost self-colour down to almost white. Castle's experiments shewed that, by selecting the darker and the lighter forms respectively, strains could be established in which the mean grade of pigmentation was in the one case considerably darker, and in the other considerably lighter than the extremes of pigmentation exhibited by the original stock¹. From his experiments Castle considered that the factor itself underwent modifications as the result of systematic selection, a view directly in opposition to that of most genetic workers, who regard the conception of the stability of the factor—relative, if not absolute—not only as an invaluable tool for progressive construction, but as firmly based upon experimental fact. The difference between Castle and his critics, however, need no longer be insisted upon, since, in his most recent contribution², he has definitely retired from the position which he formerly took up. He admits that the difference between a self-coloured rat and a hooded rat, whether of the highly pigmented or of the nearly white form, is a difference of a single factor: and further that this factor is not influenced by systematic

¹ The "original stock" consisted of relatively few individuals, and those probably with markings near what is demanded by the fancy in a hooded rat, i.e. about midway between the extremes of dark and light. Had it consisted of some thousands, allowed to breed together promiscuously without interference on the part of the fancier, it would probably have exhibited forms comparable with the extremes of dark and light eventually attained by selection.

² Castle, W. E., *Studies of Heredity in Rabbits, Rats, and Mice*. Publ. No. 288, Carnegie Inst., Wash., 1919.

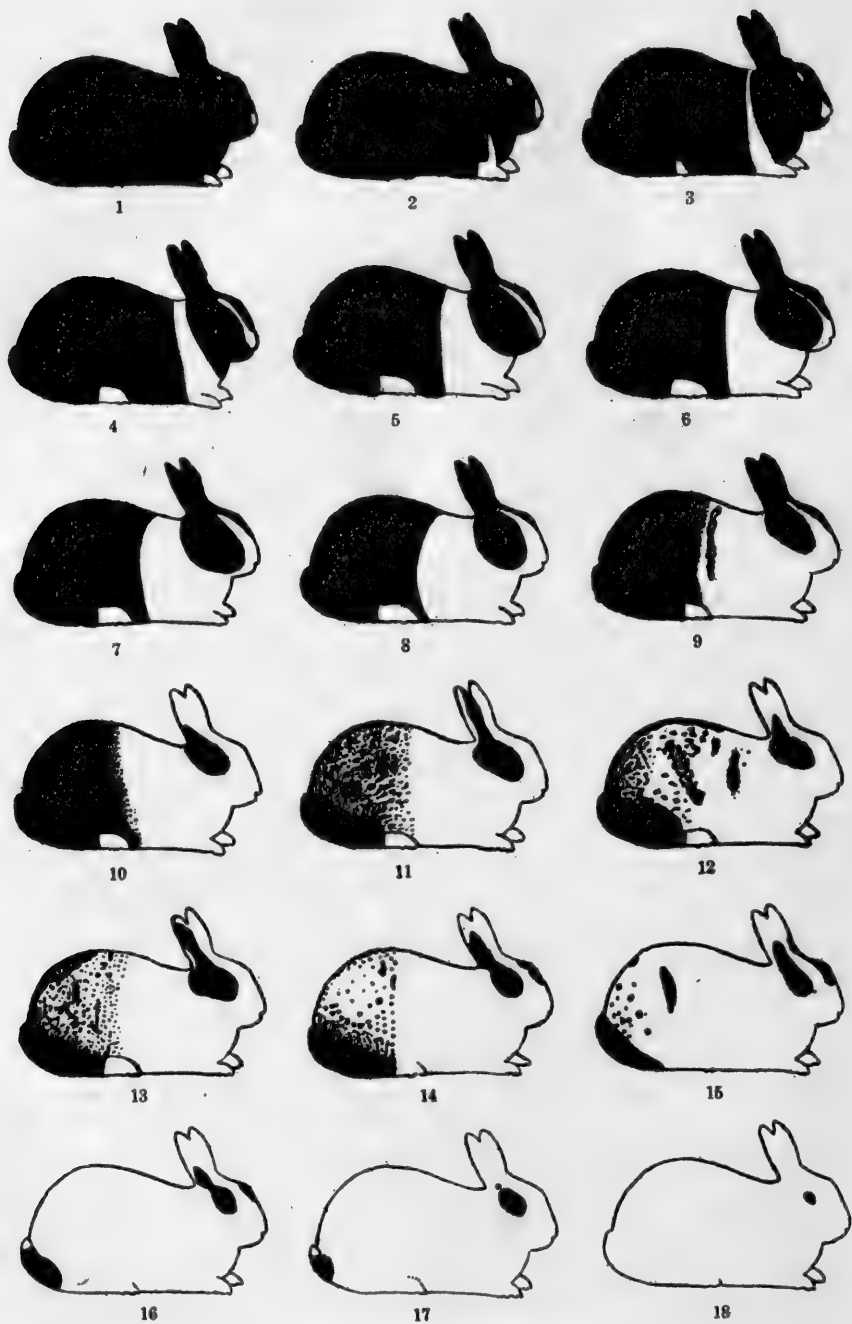
selection as he had previously held. That the recessive hooded form may shew such a wide range of variation, from an almost fully pigmented to a nearly white type, he attributes to "residual heredity." This term, so far as I can understand it, I take to mean practically the same thing as the "modifying factors" of Morgan and other critics of Castle. When, by selection, a markedly darker strain of hooded rats is formed from average individuals, we may suppose that a larger proportion of factors whose modifying activity is towards fuller pigmentation has been accumulated in that strain, the members of the strain remaining unchanged in respect of the factor that determines the difference between self-colour and the hooded pattern. If this is what is meant by saying that an alteration in "residual heredity" has taken place in these selected rats, it is clear that Castle's position is brought into line with a strict factorial interpretation of the continuous series of grades of pigmentation exhibited by hooded rats. The next step in the analysis of this celebrated case is obviously to attempt to define the number and scope of these postulated modifying factors, an experimental task which is likely to demand a very considerable expenditure of time and labour.

Meanwhile we may consider Castle's other series of experiments with an animal shewing an apparently continuous series of graded patterns, viz. that dealing with Dutch rabbits, of which an account has just been published¹. I am led to discuss this series in some detail because I have been investigating the case for the past twelve years², and while in general my results are in accordance with those obtained by Castle I have been led to adopt an interpretation different from that which he has just put forward. My experiments are not yet complete, and probably several years must elapse before I am in a position to present a full account. Nevertheless I have gained sufficient experience to venture upon a tentative interpretation, and I feel that, in the interest of the case itself, as well as of other workers on similar lines, it would be of service if I put it forward in conjunction with the data presented in Castle's valuable memoir.

In order to facilitate discussion I have taken the liberty of reproducing one of the plates accompanying his paper. On this (Fig. 1), as will be seen, Castle arranges his animals in 18 grades with an almost self-coloured animal at one extreme (grade 1), and at the other a white

¹ *Op. cit.*

² My experiments were started in 1907. From 1911 till the outbreak of war I was helped by the late Major P. G. Bailey. Since then I have continued to carry them on alone.



Grades 1-18 of Dutch rabbits.

Fig. 1.

animal with pigmented eyes (grade 18). This last form does not appear to have occurred in Castle's experiments¹, nor has it turned up in my own, though both of us have met with animals figured as grade 17.

As the result of his experiments Castle distinguishes four types, viz.:

(1) *Self-colour*, without any trace of white.

(2) *Dark Dutch*, in which he includes animals of grades 1-7.

(3) "*Tan*" *Dutch*, a form of Dutch originating from a cross between yellow Dutch and Black-and-tan. The members of the strain varied between grades 2-5, the great majority belonging to grades 3 and 4. The grades used in describing these animals are the same as those used for the Dark Dutch. There is however a qualitative difference in that a Tan Dutch has more white on the nose and blaze with less on the collar than the corresponding grade of Dark Dutch. The character is probably that described some years ago as the "New Style" Dutch², to distinguish it from the "Old Style" which is the typical animal of the fancy.

(4) *White Dutch*, which may be of grades 15-17.

These four types are regarded by Castle as due to a system of multiple allelomorphs. Animals with a pattern grade of 8-14 would appear to be regarded either as heterozygous forms, or as due to "mutual modification" by which "the several members of this allelomorphic series tend, as a result of crosses, to become more like one another." Such "mutual modification" Castle states "need not be regarded as change in the nature of a single gene, but merely as equalization of the residual heredity additional to the single genes which produce monohybrid ratios³." At the same time Castle does not appear to be quite clear whether "mutual modification" will account for the appearance of all of the various grades that are not covered by his four type forms, for he states that there may be "possibly many other types or conditions of white-spotting which with sufficiently accurate observation might be distinguished from each other⁴": though whether he intends this to apply to forms which appeared in the course of his experiments, or whether he is referring to the possibility of other forms appearing which have so far not been met with, is not clear.

What we want at present to determine in connection with these cases of continuous series is whether they can be expressed satisfactorily

¹ A white rabbit with pigmented (blue) eyes is however known: cf. *Einführung in die experimentelle Vererbungslehre*, von E. Baur, 2 Aufl. Berlin, 1914, Fig. 28a, p. 75.

² *The Book of the Rabbit*, by L. Upcott Gill, 1881, p. 51.

³ *Op. cit.* p. 19.

⁴ *Op. cit.* p. 18.

on a strictly factorial basis: whether we can assign to each member of the series a definite genetic constitution, the validity of which we can test by definitely devised experiment. To some extent Castle's interpretation is a factorial one in that he offers an explanation in terms of the four factors forming his system of multiple allelomorphs. He admits however that some of the data can hardly be brought into line with his interpretation, a point to which I shall recur later. On the other hand, by postulating "mutual modification" between members of his allelomorphic series he appears to me to be introducing a complication without the justification of fact. I confess however that I am not clear as to the nature of the process involved in this term. It must be something distinct from heterozygous forms, because it involves the conception of the several members of the allelomorphic series tending, as the result of crosses, to become more like one another. Yet we are told that it "need not be regarded as change in the nature of a single gene, but merely as equalization of the residual heredity additional to the single genes which produce hybrid ratios¹." So far as I can understand this it appears to be an unnecessarily complicated and round-about way of saying that there are other factors concerned of which the nature has not yet been demonstrated experimentally. I propose therefore to dismiss the terms "mutual modification" and "residual heredity" from the discussion for the present, and to enquire whether we cannot frame a simpler hypothesis to cover the facts than that adduced by Castle.

Before doing so, however, I wish to draw attention to several points in connection with which Castle's hypothesis appears to me to be unsatisfactory. In the first place before we can offer an interpretation on factorial lines we must be in a position to present evidence for the existence of the various homozygous forms which that interpretation involves. We must determine by experiment which of the terms of our apparently continuous series can be got to breed true, and which cannot. By breeding true is not of course meant that any individual in a homozygous strain should be an exact replica of any other. For reasons that are at present beyond us, the individuals of a homozygous strain may be expected to present some amount of fluctuation in the extent of the white markings. This however need not be very great.

¹ It would appear from a sentence on p. 16 that Castle does not bind himself by this admission, for he states his opinion here "that dark and tan are allelomorphs but separate in a modified form, one on the whole darker the other on the whole lighter than before they were crossed with each other."

Castle's Tan Dutch for example were almost all of grades 3-4, and their variation did not extend beyond grades 2 and 5. Again the members of his White Dutch strain all fell into grades 15-17, and in the course of my experiments I have also established a strain in which the variation is about the same. I think we may fairly regard these as homozygous strains. But when we turn to the Dark Dutch, varying from grades 1-7, I feel that the position is different, and I am not prepared to classify together under the same head animals which are almost self-coloured and others which might pass for the typical Dutch of the fancy. It is quite possible, as will appear later, that among Castle's Dark Dutch several strains could be got to breed approximately true to different grades of pigmentation. My own data point to the fact that it is possible to obtain a strain of Dutch breeding true to about grades 7 and 8. If this is substantiated by further experiment it introduces a new factor into the case. Further, I am inclined to believe that it is possible to obtain a strain which breeds true to about grades 11-13. Here again my experiments are not yet complete, for larger numbers are required before this point can be regarded as established. For the present I look upon the following as weak points in Castle's interpretation, viz. (a) the inclusion of grades 1-7 in a single category, (b) the non-recognition of a true breeding strain of typical Dutch (grades 7-8)¹, and (c) the non-recognition of a true breeding strain of Spotted Dutch varying about grades 11-13.

The interpretation that I wish to put forward provisionally is as follows. We may suppose that there are several pattern forms with reduced pigmentation which breed approximately true, viz.

- (a) *White Dutch*, comprising animals of grades 15-17.
- (b) *Spotted Dutch*, varying about grades 11-13.
- (c) *Typical Dutch*, varying about grades 7-8.

¹ Castle regards these grades as generally, if not always, heterozygotes between Dark and White Dutch (*op. cit.* p. 7), and this is doubtless very often true. Nevertheless I think it equally beyond a doubt that such animals may be homozygous. Indeed Castle probably used such an animal in his experiments. Of the original two Dutch bucks he started with one (σ 3036) was from his breeding behaviour obviously heterozygous. But the evidence given by Castle strongly suggests that the other (σ 3037) was not carrying White Dutch. There is no record of his being mated directly to White Dutch, but five of his F_1 daughters from self-coloured mothers were mated with σ 3036 (*op. cit.* Tables 14 and 16) and produced 50 offspring, all of grades 1-9. Had σ 3037 been heterozygous it is most likely that some of his tested daughters would have carried White Dutch, and would, when mated with the heterozygous σ 3036, have produced some offspring with a grade of pigmentation approximating to White Dutch.

With regard to (a) I agree with Castle that a true breeding strain can be readily established, and I have had one in my possession for some years. With regard to the establishment of (b) and (c) my experiments are not yet complete, though at the same time the evidence I have accumulated supports the suggestion made above. It is however possible that another true-breeding form of Spotted Dutch exists varying about grade 14, but at present my data are not sufficient to justify the assertion of its existence. Moreover in the case of the Typical Dutch (c) I have not yet succeeded in obtaining a strain in which the pigmented area is entirely free from white in every individual. Though the area of pigmentation varies about that shewn in grade 7, many individuals shew small blurred patches of white on the dorsal surface (cf. Pl. XI, Fig. 1). Whether a strain of Typical Dutch can be obtained without these small patches is a question now under investigation. In any case the evidence is not sufficient to connect these small patches definitely with the Spotted and White Dutch characters.

Further, I should mention that Typical Dutch animals may be heterozygous for Spotted Dutch, though perhaps the grade of pigmentation is somewhat lower in the heterozygote than in the homozygote. Crossed with White Dutch however both Typical and Spotted Dutch give rise to intermediate forms. The F_2 generation exhibits segregation, the parental types reappearing together with intermediates. The cross between White and Typical Dutch for example produces F_1 animals indistinguishable in appearance from Spotted Dutch (cf. Pl. XI, Figs. 3 and 4). Genetically however they are distinct, for such F_1 animals bred together have produced among their progeny both White and Typical Dutch.

Assuming then the existence of the three strains (a), (b), and (c) we may turn to the higher grades of pigmentation, viz. grades 1-6. My experiments have yielded evidence for the existence of a factor (P) which, when added to any one of the three forms (a), (b), or (c) greatly increases the development of the pigmentation. When added to the White Dutch for example it brings up the pigmentation to a grade characteristic of Typical Dutch¹; when added to Spotted Dutch, animals approximating to grade 3 result; and when added to Typical Dutch it leads to the production of animals which are almost, or even quite self-coloured. An interesting point in connection with P is its relation to the colouration of the iris. Dutch rabbits, particularly those of a low

¹ It is interesting here to recall that Baur obtained F_1 animals near Typical Dutch on crossing his blue-eyed whites with albino; cf. *Einführung in die experimentelle Vererbungslehre*, 1914, p. 75.

grade, such as Spotted and White Dutch, often exhibit heterochromia iridis. In the lowest grades (i.e. those with most white) this may not infrequently extend so far as to result in completely pale blue, or "wall" eyes¹. Even in typical Dutch, cases occur in which one or both eyes are blue², though more frequently the iris is patched. I have hitherto met with no case of heterochromia in a rabbit which contains **P**. A White Dutch, to which **P** has been added, is of a grade of pigmentation very like that of a Typical Dutch, and its eyes are normal brown. When such animals, heterozygous for **P**, are mated back to White Dutch, they produce approximately equal numbers of White Dutch and of animals near Typical Dutch in appearance. Of those that I have so far bred in this way, the white have always been heterochromic, whereas those containing **P** have never been heterochromic. This relation of **P** to heterochromia promises to be of much assistance in the analysis of the higher grades of pigmentation.

Whether **P** produces a similar effect in a single and a double dose is a point which I have not yet been able to decide, but experiments with this object are now in progress³. The bearing of the demonstration of this factor upon the nature of the higher grades of pigmentation (i.e. those with more colour) is however sufficiently clear. Corresponding to each lower grade we must suppose that there is a higher grade, differing from the former in that it also contains **P**; and if certain lower grades breed true there is no reason why we should not eventually find a corresponding series of higher grades also breeding true. The self-coloured rabbit, the highest grade of all, I am at present inclined to regard as a Typical Dutch which is also homozygous for **P**.

Regarded from this point of view Castle's procedure of lumping all the higher grades together as Dark Dutch is justified in so far as these higher grades contain a factor which is not present in the lower grades. It does not however take into account the fact that they may vary greatly with respect to those other genetic factors to which are due the differences between Typical, Spotted, and White Dutch, and their various heterozygous forms.

With this brief outline of the interpretation I would suggest, an

¹ The White Dutch animal figured on Pl. 2, Fig. 19 of Castle's memoir evidently has a "wall" eye. So also has the white rabbit figured by Baur to which reference was made earlier.

² Cf. Bond, C. J., *Journ. Genet.* Vol. II, 1912, p. 111.

³ Evidence is accumulating that **PP** animals are more pigmented than the corresponding **Pp** forms. This has been allowed for in Fig. 2 (p. 312) except in the case of **Pp**ttss rabbits. The grade of such animals is probably about 3—5 instead of 6—8 as given in Fig. 2.

interpretation admittedly tentative in view of the present state of the analysis; we may turn to examine Castle's facts. In doing so it will be convenient to consider his different crosses separately, though not necessarily in the order in which he gives them.

(1) *The cross between Self-colour and White Dutch (pp. 10-12)*¹. A White Dutch ♂ of grade 17 was crossed with four self-coloured does, and produced 33 young of grades 1-3. An F_2 generation of 191 individuals was raised, and in it was represented every grade of pigmentation from self to grade 16. Such an F_2 generation is certainly not to be expected on the assumption that Self-colour and White Dutch belong to a system of multiple allelomorphs; and indeed Castle admits that it "is somewhat

PTS PTS 0	PTS PTs 0	PTS PtS 0	PTS Pls 0	PTS pTS 1	PTS pTs 1	PTS ptS 1-2	PTS pts 1-3
PTs PTS 0	PTs PTs 0	PTs PtS 0	PTs Pls 0	PTs pTS 1	PTs pTs 1	PTs ptS 1-3	PTs pts 2-4
PtS PTS 0	PtS PTs 0	PtS PtS 1	PtS Pls 1-3	PtS pTS 1-2	PtS pTs 1-3	PtS ptS 3-5	PtS pts 4-6
Pts PTS 0	Pts PTs 0	Pts PtS 1-3	Pts Pls 6-8	Pts pTS 1-3	Pts pTs 2-4	Pts ptS 4-6	Pts pts 6-8
pTS PTS 1	pTS PTs 1	pTS PtS 1-2	pTS Pls 1-3	pTS pTS 7-9	pTS pTs 7-9	pTS ptS 8-10	pTS pts 13-15
pTs PTS 1	pTs PTs 1	pTs PtS 1-3	pTs Pls 2-3	pTs pTS 7-9	pTs pTs 10-12	pTs ptS 13-15	pTs pts 14-16
ptS PTS 1-2	ptS PTs 1-3	ptS PtS 3-5	ptS Pls 4-6	ptS pTS 8-10	ptS pTs 13-15	ptS ptS 11-13	ptS pts 14-16
pts PTS 1-3	pts PTs 2-4	pts PtS 4-6	pts Pls 6-8	pts pTS 13-15	pts pTs 14-16	pts ptS 14-16	pts pts 16-17

Fig. 2.

¹ References given in italics are to Castle's paper.

puzzling in character." It is however what we should expect on the hypothesis that I have outlined above, and since this case offers a good test between Castle's interpretation and my own we may examine it in more detail.

Let it be supposed that the self-coloured rabbit is homozygous for the three following factors:

P, the factor for increased pigmentation (as explained above).

T, a factor which in the presence of **S** leads to the production of a Typical Dutch. We may further suppose that when **S** is not present **T** leads to some increase in the pigmentation of the White Dutch.

S, a factor which, when added to White Dutch, leads to an increase in the pigmentation, and the formation of the Spotted Dutch character. The heterozygote **Ss** is intermediate in form.

The F_1 rabbits ex Self-coloured \times White Dutch will be **PpTtSs** in constitution, and will give rise to the series of gametes **PTS**, **PTs**, **PtS**, **Pts**, **pTS**, **pTs**, **ptS**, **pts**. The zygotic series produced by two such series of gametes is represented on Fig. 2, on which are also indicated the grades to which the various zygotes may be referred. It will be understood of course that any rigid system of assignment is impossible; for, after all, the grades made use of are but convenient symbols. In making the various assignments I have been guided as far as possible by the experience gained through many varieties of mating extending over a period of more than ten years. In Table I the various grades have

TABLE I.

Grades	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Distribution calculated from Fig. 2	36	43	20	16	10	6	7	6	8	5	3	2	2	5	8	8	6	1
Actual figures from Castle	41	45	22	14	9	5	5	4	5	5	8	4	8	5	6	4	1	—

been collected together from Fig. 2, and brought into tabular form in order that the hypothetical result may be compared with Castle's actual figures¹ (*Table 28, p. 45*). The general agreement is evidently close, and it is clear that the experimental data are more in accordance with the interpretation I have suggested than with that which Castle has put forward.

¹ The number of rabbits in Castle's F_2 generation is 191; accordingly I have multiplied each zygote in Fig. 2 by 3, bringing the total to 192. Where a zygote is given as of several adjoining grades each is represented in Table I. Thus where a zygote is given in Fig. 2 as of grades 4-6, I have reckoned one of each of these three grades in Table I.

Further data of Castle's are those obtained by mating F_1 animals with a White Dutch (*Text-fig. 2, p. 11*). The 116 offspring fall into two groups without overlapping, each containing 58 individuals. On the hypothesis of multiple allelomorphs we should have expected the group with greater pigmentation to have shewn a distribution of grades similar to F_1 animals. As a matter of fact the mean grade is 5-6, and the range between 1 and 9, whereas in the F_1 animals the range is only 1-3, with a mean about 1.5. So also in the light groups the range is 13-17 as against that of 15-17 in White Dutch, while the mean is about 15 as against between 16 and 17 in White Dutch. This extension of the ranges and shifting of the means of the two groups is however what is to be expected on the interpretation I have suggested. Making use of the grades assigned in Fig. 2 to the various genetic constitutions involved, I have worked out the distribution of the progeny for this cross, as shewn in Table II. Here again, as in the case of the F_2 generation, the hypothesis I have suggested appears to fit the facts better than that of multiple allelomorphs with its necessary concomitant of "mutual modification."

TABLE II.

Grades	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Calculated Distribution }	5	10	10	10	5	10	5	5	—	—	—	—	—	10	10	15	17	7
Actual distribution from Castle }	2	3	4	11	11	14	9	3	1	—	—	—	—	1	14	24	13	6

(2) *The cross between Dark Dutch and White Dutch (pp. 8-10).* In this cross five ♀♀ of grades 6-8 were mated with a ♂ of grade 17. The majority of the offspring were of grades 5-9 (*Text-fig. 1, p. 9*), though two were of grades 10 and 11 respectively. As both of these were from the same doe (♀ 6666, *Table 22*) it suggests that she may have been heterozygous for P, while the other four were homozygous. A true F_2 generation was apparently not raised, but animals of grades 4-9, variously produced and known to throw the White Dutch form, were bred together. The 56 progeny resulting varied between grades 1-17. They may be divided into two groups, (a) with a range between grades 1-10, and (b) a smaller group with a range of 12-17. The mean grade of (a) is higher (i.e. less pigmented) than that of the dark strain as a whole, while the mean of (b) is lower than that of the white strain as a whole. In other words the two groups into which this " F_2 " generation falls approximate more closely to one another than do the parental dark and white groups

(*Text-fig. 1, p. 9*). If the dark and light belong to a system of multiple allelomorphs segregating in the normal way we should not expect this approximation, and I gather that it is in explanation of it that Castle suggests the hypothesis of "mutual modification." On the interpretation I have suggested there is no need to postulate any such problematical process. The appearance of such grades as 9-14 in the " F_2 " generation may be supposed to be due to the fact that some of the parents, especially those belonging to the higher numbered grades, were heterozygous for **S** as well as for **P**. From such animals bred together, on the assumption that normal and independent segregation of the several factors occurred, we should expect various intermediates of grades 9-14. Further we should also look for a small proportion of animals tending towards complete pigmentation. For such might be expected to occur when **P** reacted on a basis of Typical Dutch. On Castle's explanation in terms of multiple allelomorphs, particularly when the hypothesis of "mutual modification" is superadded, it is difficult to understand why these highly pigmented rabbits should appear in the " F_2 " generation.

The result of mating F_1 (ex Dark \times White Dutch) with White Dutch was the production of two groups of about equal size, but slightly overlapping (*Text-fig. 1, p. 9*), so that every grade between 4 and 17 was represented. On the assumption that the dark and light belong to a system of multiple allelomorphs it was to be expected that the two groups would be sharply separated, and that their mean grades would be close to those of the parental stocks. Here again Castle finds evidence for "mutual modification," but, as in the case of the " F_2 " generation, there is no need for this assumption on the interpretation that I have suggested.

(3) *The cross between Tan Dutch and White Dutch (pp. 13-14)*. From this cross the F_1 individuals were intermediate in grade ranging from 6-9, the range of Tan Dutch being 2-5, and that of White Dutch 15-17. The F_2 generation comprising 130 individuals, forms a continuous and almost regular curve on grading, with the mode very close to the mean, and with a range of 3-14. Animals ex $F_1 \times$ White Dutch exhibit a range of 7-17. All of these results are in accord with the supposition that Tan Dutch and White Dutch differ in several factors from one another, such factors being all present in Tan Dutch but absent from White Dutch. Castle supposes that Tan Dutch belongs to the same multiple allelomorph system as Self-colour, White, and Dark Dutch. The experimental facts appear to me to be entirely opposed to

such an interpretation, even when we make a most generous allowance for "mutual modification." The absence of selfs in F_2 is an argument used by Castle in favour of his view; and though I agree that both Tan and White Dutch must lack something present in the self-coloured animal, I fail to see that this entails the creation of a series of multiple allelomorphs to represent these three states of pigmentation.

I am inclined to regard the Tan Dutch as a definite stage of higher pigmentation in the series White-Spotted-Typical Dutch, and consequently lacking P. This view receives some support from a piece of information which Professor Castle very kindly sent in answer to an enquiry of mine, i.e. that Tan Dutch rabbits may exhibit heterochromia iridis. If we denote as N the factor that turns a Typical Dutch into a Tan, or New Style, Dutch (cf. p. 307), the cross Tan Dutch \times White Dutch would result in F_1 heterozygotes of the constitution $NnTtSs$, and these might be expected to give results similar to those which Castle obtained, both when mated together and when crossed back with White Dutch.

(4) *The cross between Dark Dutch and Tan Dutch (pp. 13-16).* Two outstanding features are here exhibited. In the first place the F_1 animals are of distinctly higher pigmentation than either of the parental forms, some of them being even self-coloured. Secondly of the F_2 generation, consisting of 275 individuals, about three-quarters are of grades 0-3 (as many as 89 being self-coloured), while the remaining quarter extends over grades 4-11. Here again the facts appear to me to be entirely opposed to the interpretation in terms of multiple allelomorphs advocated by Castle; for it offers no explanation of the self-coloured animals in F_1 , or in F_2 where they constitute about 30% of the total. Nor does it explain the considerable range in F_2 , extending so far as grade 11. On the interpretation I have suggested these facts are intelligible. The Dark Dutch animals used varied from grades 2-6. Consequently, though homozygous for P, they may well have lacked either T or S, or in some cases both. The F_1 animals would be heterozygous for P and N, and this would entail their being self-coloured, or nearly so. But at the same time if lacking in T or S they would be constitutionally $PpNnTtSS$, or $PpNnTTsS$, while some might be $PpNnTtSs$ ¹. From such animals we should expect an F_2 generation similar to that which Castle obtained.

This cursory examination of Castle's data is sufficient to make it clear that the hypothesis of multiple allelomorphs advocated by him

¹ Probably some were of this constitution, for when crossed with White Dutch a few animals of grades 16 and 17 appeared (cf. *Text-fig. 5*, p. 15).

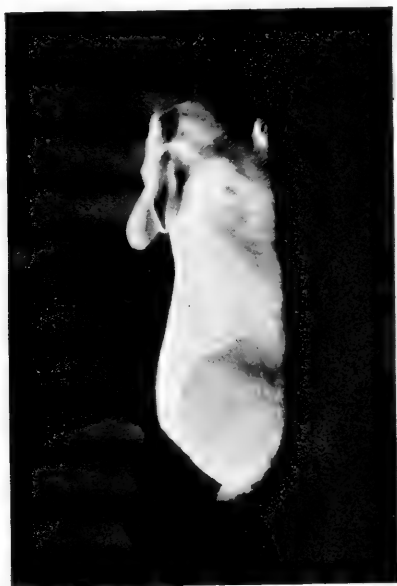


Fig. 2.



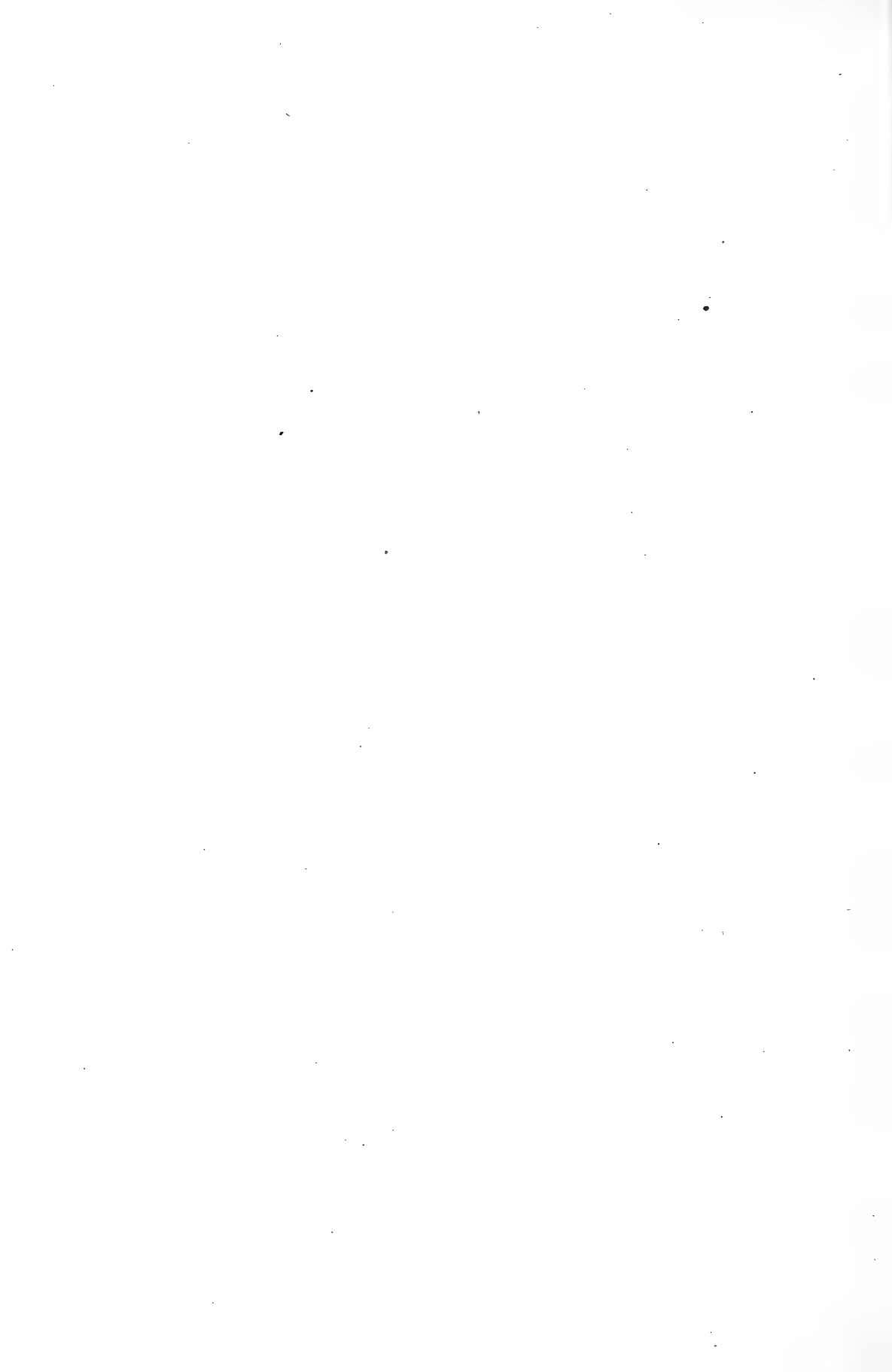
Fig. 4.



Fig. 1.



Fig. 3.

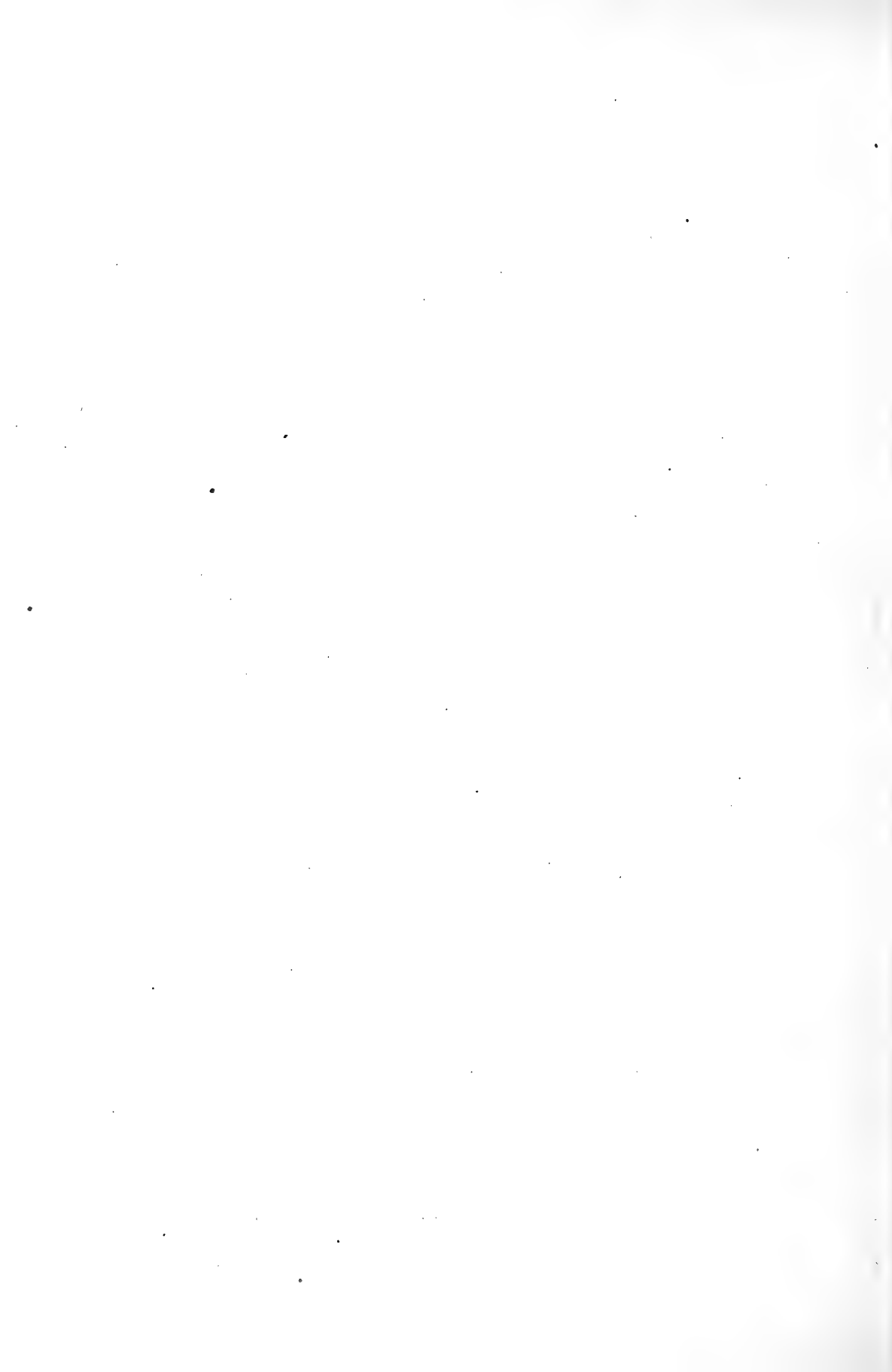


is a far less satisfactory explanation of the facts than the hypothesis of independent factors such as I have suggested. Moreover on this latter hypothesis there is no need to drag in any conception of "mutual modification." Such a conception is in my opinion entirely opposed to the general trend of modern genetic work. If, after adequate analysis, cases are found which afford indubitable evidence for such a process, then it must be carefully taken into account. But after many years spent in breeding Dutch rabbits I am strongly inclined to believe that the facts will eventually receive an explanation on a strictly factorial basis; nor at present do I see good reason for supposing that the factors concerned shew any more tendency to undergo modification through crossing than in the countless instances in both plants and animals where apparent clean segregation is the rule.

[The experiments on Dutch rabbits alluded to in this paper have been carried on with assistance from the Development Commission Fund administered by the Board of Agriculture. For those dealing with heterochromia iridis grants have been received from the Government Grant Commission.]

EXPLANATION OF PLATE XI.

- Fig. 1. Typical Dutch rabbit. The right eye shews some heterochromia. Note the small white patches on the back.
Fig. 2. White Dutch. In this animal both eyes were blue.
Fig. 3. Spotted Dutch. The pigmentation in most animals of this strain is rather more pronounced.
Fig. 4. F_1 rabbit ex Typical Dutch \times White Dutch.



THE TYPES OF SPOTTING IN MICE AND THEIR GENETIC BEHAVIOUR¹.

BY MASAO SÔ AND YOSHITAKA IMAI.

(With Plate XII.)

CONTENTS.

	PAGE
Introduction	319
Material used	321
Mating: Self \times Piebald	321
Mating: "Kasuri" \times Piebald	322
Mating: "Kasuri" \times Self	323
The "Kasuri" Race carrying Self Factor in the Homozygous Condition	324
Mating: "Daruma" \times Piebald	325
Mating: "Daruma" \times "Daruma"	326
The Modified Ratio	326
Mating: "Daruma" \times Self	327
Mating: "Kasuri" \times "Daruma"	328
Mating: "Kasuri" \times "Kasuri"	328
The Behaviour of the D Factor	329
Extracted Self and Piebald	330
Explanation of Plate	332
Literature cited	332

Introduction.

THE manifold types of the coat pattern in mice as well as in other rodents have furnished convenient material for genetic investigation. Spotted or piebald type is a well-known variation in these animals. Utilizing this form in breeding experiments, the results obtained by different authors arrive at the same conclusion, i.e. on the whole the spotting behaves as a recessive to the self-coloured.

In rats the type of the spotting is somewhat limited as it hardly varies beyond the limits of the hooded marking. On the other hand,

¹ A preliminary paper (in Japanese), with a description on the inheritance of albinism in mice, was published in the *Journal of the Scientific Agricultural Society*, No. 187, 1918.

in mice and in guinea-pigs it varies so much, both in amount as well as in type, that there exists hardly any definite, well-recognized type such as hooding in rats. Furthermore, it has often been stated that the variation of the spotting in these animals not only occurs in a continuous series of gradations, but also extends from the nearly self-coloured animals to one having a slightly pigmented coat.

In mice, besides the recessive spotting just stated above, there was recognized the existence of a so-called dominant spotting, as well as a dark-eyed white which carried a special factor.

Miss Durham (1908) seems to be the first author who took these special races for use in breeding experiments. Crossing such a dark-eyed white animal to the self-coloured one she obtained the so-called dominant piebald, which, however, she failed to distinguish phenotypically from the ordinary recessive one. In that paper she stated incidentally that when such a dominant piebald is mated with itself the result may be expected to be spotted and self-coloured young in a ratio of three to one; or, in other words, such a spotting is transmitted as a dominant Mendelian unit to the self, which, in turn, dominates over the ordinary spotting. Actually, however, the case is somewhat complicated, as will be seen in the following sections. As to the significance of the dark-eyed white she did not pay much attention to it.

Morgan (1909) and Hagedoorn (1912) seem to have taken these types in their breeding experiments, but the evidence obtained by them was not enough to be convincing.

With these works already known the writers took up the experiments in 1915 hoping, if possible, to understand their real nature. But, at the close of that year, Little's important paper (1915) was published, in which he gave an account in full of this subject. He detected another independent factor for the coat pattern, in addition to those for self and common piebald. In the following year Detlefsen (1916) made similar observations with special emphasis on the pink-eyed white carrying the colour factor (= pink-eyed "Daruma"). Recently, Little (1917) attempted to find out the relation of the lethal action between the factors of black-eyed white and yellow coloured.

The results obtained by the writers agree, on the whole, with those reported by Little and by Detlefsen, but in the present paper they hope to give some additional facts on this pattern inheritance in mice.

All the authors cited above failed to separate phenotypically the two types of spotting and recorded them together in the mixed progeny, though they are entirely different genetically one from the other. In

the present investigation the distinction between them was made without marked difficulty. Some authors adopted the method of measurement of the per cent. of pigmentation of the dorsal surface in classifying the types of spotting. The writers, however, made the classification depend upon the markings proper to the races. This method is much simpler and gives very satisfactory results.

In the present paper the dark-eyed white carrying a special factor will be called "Daruma¹," while for Miss Durham's dominant spotting, or Little's spotting "Type A," the writers will use our word "Kasuri²."

MATERIAL USED.

Through the kindness of Mr Mito, a fancier in Tôkyô, the material used in the present investigation was mainly obtained. As these mice belonged to the Japanese breed "Nankin," which has not only smaller size of body but less resistance towards disease and unfavourable environment compared to the European ones, the writers paid much attention to improving its health by crossing with the common breed of the latter.

Mating: Self × Piebald.

The common piebald mice have long been used in breeding experiments, and its recessive nature to the self-coloured is already proven by many authors. The writers also repeated the experiment and secured the same results.

The young born in this sort of mating, however, were often neglected and unrecorded in the present study, so the numbers contained in this section are only a part of the results obtained in these matings. The animals used in this cross were introduced from other fanciers; those who have not specially bred either the "Kasuri" or the "Daruma" race.

When the normal piebalds are crossed with selfs the F_1 animals are all self-coloured. In the next generation, however, inbreeding of these hybrids produces offspring consisting of self and piebald in the proportion of almost three to one. The actual numbers raised in the F_2 generation from this type of mating are represented in the following table:

		Self	Piebald	Totals
Observed	...	42	16	58
Expected	...	43.5	14.5	58

¹ "Daruma" or more correctly "Yuki-Daruma" means snow-man in English. The name is given because the animal has a white coat contrasting with the black eyes which Japanese children always give their snow-men.

² "Kasuri" means spotted.

322 *Spotting in Mice and their Genetic Behaviour*

If such heterozygous F_1 animals are back-crossed with selfs of pure race the resulting young, without exception, are selfs, as is expected. When back-crossed with piebalds, the expectation is equality of the ratio of the two forms, namely, one self to one spotted. The actual results thus obtained are represented in the following table:

		Self	Piebald	Totals
Observed	77	82	159
Expected	...	79.5	79.5	159

An inspection of the above-mentioned data shows that the results entirely agree with those obtained by other investigators, the spotting being a recessive Mendelian unit to the self-coloured.

Mating: "Kasuri" × Piebald.

In attempting to separate phenotypically the "Kasuri" race from the ordinary piebald, none of the authors distinguished it clearly, though they were aware of the special silvered markings of the former type. In the study made by the present writers, however, the distinction between these two races was made without any great difficulty. Actually the "Kasuri" pattern is a sort of spotting, but this form is characterized by fine silvered markings. With this appearance kept in mind the two types of spotting may easily be separated from each other, though in a few cases the former pattern fails to develop to its markings. But such a trouble is not a matter confined only to this case, it is almost equally met with in the case between "Kasuri" and self, "Daruma" and "Kasuri," and "Daruma" and piebald as well as between self and piebald. In these doubtful cases, of course, the breeding test serves as a sole key by which to know their real nature.

To understand the genetic composition of the "Kasuri" race the writers often made the cross "Kasuri" × piebald reciprocally, for the latter animal carries the pattern factors in the lowest condition. Neither parent is self-coloured but has a sort of spotting, while among the offspring of these matings there appeared many selfs. Now, such extracted selfs must be attributed in their origin to the "Kasuri" parent, for no other carries the self factor, as was shown in the last section. And as each sort raised is in almost an equal ratio the results are similar to that when the double heterozygote is back-crossed to the double recessive one in Mendelian inheritance. The following allelomorphs assumed may suffice to illustrate these phenomena:

I. A factor, **S**, is responsible for self and its allelomorph **S'** stands for spotting of recessive nature.

II. A factor, **D**, acts as epistatic upon the former allelomorphs in the following ways: When the animal carries **S**, **D** works upon this, and the development of the pigment on the coat is restricted to a sort of spotting resulting in the "Kasuri" pattern; when **S'** takes the place of **S**, the pigment is almost entirely inhibited its development except as to the eyes. And **d** (an alternative mate of **D** factor) may be regarded apparently as having no influence upon its hypostatic allelomorphs, **S** and **S'**.

If the assumption is correct that the "Kasuri" race used in these matings was doubly heterozygous as to the allelomorphs above represented, every form resulting among the offspring may be expected in almost an equal ratio in every cross, for the genetic composition of the piebald is invariably **S'S'dd**.

The crossings of this sort were made reciprocally, but no marked difference is detected between them. In the following table there are given the totals of the reciprocal results obtained from such a sort of mating:

		"Kasuri"	"Daruma"	Self	Piebald	Totals
Observed	...	100	87	118	118	423
Expected	...	105.75	105.75	105.75	105.75	423

On the whole the numbers obtained agree to the expectation, though there is observed some marked discrepancy of the ratio.

Mating: "Kasuri" × Self.

When such a doubly heterozygous "Kasuri" race is crossed with self the results expected are different, depending upon the genetic composition of the latter animal. If homozygous selfs are used in this sort of mating the offspring consist of "Kasuri" and self in almost equal numbers, the actual results being as follows:

		"Kasuri"	Self	Totals
Observed	...	111	133	244
Expected	...	122	122	244

The deviation from equality is somewhat large, and may be either a chance deviation, or the consequence of the selective mortality among the young, as it was often conspicuously observed in this sort of mating.

When the heterozygous selfs, however, are mated with this race the expected ratio is a 3:1:3:1 ratio of "Kasuri," "Daruma," self, and piebald respectively. In the following table are represented the results obtained:

		"Kasuri"	"Daruma"	Self	Piebald	Totals
Observed	...	104	39	94	52	289
Expected	...	108.375	36.125	108.375	36.125	289

324 *Spotting in Mice and their Genetic Behaviour*

A marked discrepancy of the ratio again appears in this case; as to the reasons for such a result the writers cannot say, but think it to be a chance deviation.

The "Kasuri" Race carrying Self Factor in the Homozygous Condition.

All of the specimens of the "Kasuri" race received from Mr Mito proved, by the breeding test, to be doubly heterozygous, i.e. having the genetic composition $SS'Dd$. If such mice are mated with selfs of the homozygous race having the constitution of $SSdd$, we should expect half the number of the "Kasuri" young born to be homozygous in regard to the self factor; while we should expect one-third in the cross "Kasuri" \times "Kasuri," and "Kasuri" \times heterozygous self or its reciprocal. But there was often observed high mortality among the "Kasuri" young born in these crosses, and, further, if they attained sexual maturity frequently they fell into a sterile condition, more markedly on the female side. Such an unfortunate occurrence, however, has been gradually lessened in the course of the experiments. Probably this is connected with an improvement in health by crossing with the strong European breed, rather than with the elimination of the lethal factor or the like. At present such sterility occurs occasionally, especially in the female, and this is possibly one of the characteristics of the "Kasuri" race. The case is somewhat similar to that observed in yellow mice.

The sterility in the yellow mice seems connected with the abnormal development of the fat tissue, but the case is somewhat different in the "Kasuri" race. In the latter they often waste away gradually and at last become sterile, though in some cases they grow quite plump like the yellow mice. These conditions are also found, in a few instances, in the "Daruma" race.

Now we shall turn to the discussion of the experimental data on the subject of this section.

Out of seven "Kasuri" males obtained from the cross "Kasuri" \times homozygous self or its reciprocal, five proved to be homozygous as to the self factor, and the rest (two) were heterozygous: of seven females having a similar origin, one was homozygous and three were heterozygous, but the rest (three) were doubtful, for the litters they gave were not sufficient to decide their genetic composition. Out of twenty-one males coming from the cross heterozygous self \times "Kasuri" only one was proved to be homozygous and eighteen were heterozygous, but the rest (two) were doubtful: of nine females of the same origin, one was homozygous and six were heterozygous, but the rest (two) were doubtful.

And out of five males coming from the cross "Kasuri" \times "Kasuri," one carried the self factor in the homozygous condition and three in the heterozygous one, one being doubtful: only one of the females from a similar mating gave a litter and proved to be a heterozygote.

These results almost exactly confirm the expectation, though a deficiency of homozygous mice was observed in the cross heterozygous self \times "Kasuri."

When such resulting heterozygous "Kasuri" mice are crossed with the ordinary piebald, four types of young are produced in almost equal proportions, as was shown before. If, however, the "Kasuri" race homozygous for **S** is mated with piebald, we should expect "Kasuri" and self in nearly equal numbers. The results obtained from these crosses are summarized in the following table:

		"Kasuri"	Self	Totals
Observed	59	54	113
Expected...	...	56.5	56.5	113

In this case the agreement between actual and calculated numbers is close.

Further experiments with the "Kasuri" race of **SSDd** composition were rendered difficult owing to its tendency to become sterile and its weakness.

Mating: "Daruma" \times Piebald.

As was mentioned above, the "Daruma" race normally has dark eyes, black or brown, according to the nature of the coat colour factor which the animal carries. But Detlefsen obtained a pink-eyed "Daruma" race which is almost indistinguishable from the albino in appearance. The writers also have often raised such a mouse as well as a ruby-eyed one.

The "Daruma" race has usually a pure white coat, but often it is blemished by a spot or spots appearing between the eyes, around the ears or in front of the tail, etc., and, though rarely, such spots form fine silvered markings scattered over the coat as in the whiter forms of the "Kasuri" pattern. Furthermore, in a few cases, such spots may assume somewhat large massive markings resembling the slightly spotted classes of the ordinary spotting. In these cases it cannot be decided whether the animal belongs to the "Daruma" race or not, without the test of breeding.

On crossing the "Daruma" race from various origins with the normal piebald, two sorts of young invariably result, almost in an equal

326 *Spotting in Mice and their Genetic Behaviour*

proportion. The totals obtained from such matings are represented in the following table:

		"Daruma"	Piebald	Totals
Observed	253	264	517
Expected	258.5	258.5	517

The expectation accords closely with the experimental data; the ratio of the two forms being one to one.

Mating: "Daruma" × "Daruma."

Curiously enough the "Daruma" race almost always gives mixed young when the animal is mated with the piebald. The results point to the heterozygosity of each mouse of the "Daruma" race used in these matings. If such heterozygous mice are mated between themselves we should expect "Daruma" and piebald in a ratio of three to one. The actual results, however, differed widely from this expectation, as will be seen in the following:

		"Daruma"	Piebald	Totals
Observed	161	81	242
Expected on 2 : 1 ratio	161.33	80.67	242
Expected on 3 : 1 ratio	181.5	60.5	242

The observed number comes strikingly close to the calculated one based on a ratio 2:1, while the normal expectation deviates widely from it.

Studying the same subject, Little formerly experienced also such an abnormal ratio from analogous matings. The interpretation of such a result will be fully discussed in the next section.

The Modified Ratio.

The modified ratio 2:1, which resulted in the cross "Daruma" × "Daruma," recalls instantly the similar result observed in yellow mice.

In breeding experiments with the yellow mice, Cuénot (1905) first reported the results in Mendelian terms. After him Castle and Little (1910) repeated the experiment and obtained a ratio of almost 2:1 of yellow:non-yellow in the cross yellow × yellow when their data are added to Cuénot's, and this was confirmed by work done by Durham (1911), Little (1911), Hagedoorn (1912) and Dun (1916). The writers breeding this race on a small scale observed also the same facts. The views of many of the authors cited above agree that the modified ratio is due to the mortality of the homozygous yellows which

occurs before their birth, though at that time they did not prove it by direct evidence.

Recently Kirkham (1917) and, independently, Ibsen and Steigleder (1917), however, have confirmed this view from their embryological studies, where the homozygous yellow mice were expected to perish. They brought forward evidence of marked mortality among the embryos, especially in the early stage or soon after the eggs attached themselves to the mother's uterine membrane.

As was indicated above, the modified ratio obtained in the "Daruma" \times "Daruma" cross is entirely similar to that of the yellow mice. According to normal Mendelian expectation one-third of the "Daruma" young born in the cross "Daruma" \times "Daruma" may be expected to be homozygous in regard to the **D** factor. So far as the writers have tested such animals, however, none of them proved to be homozygous. Thirty of them were used for this purpose, and all of them were found to carry the factor in the heterozygous condition. Formerly Little, also testing twenty-one of them coming from a similar origin, obtained the same results. If this number is added to the writers' figure we may safely conclude that the "Daruma" mouse carrying the **D** factor in the homozygous condition is either never born or matured. It may be possibly thought that as the mortality occurs soon after birth, yet before the time at which the pattern character can be recorded, one-third of the "Daruma" mice meeting with such a fate may not be actually recognized. To answer this question the original record was carefully examined, but the data led to the negative conclusion. So there seems to be no mistake in saying that the homozygous "Daruma" race do perish at the embryological stage like the yellow mice, though direct evidence supporting this view is not in hand.

Mating: "Daruma" \times Self.

When homozygous selfs are mated with the "Daruma" race we should expect equal numbers of "Kasuri" and self in the subsequent generation, as the latter parent is invariably of heterozygous genetic composition. The actual numbers obtained from these matings are represented in the following table:

		"Kasuri"	Self	Totals
Observed	...	19	15	34
Expected	...	17	17	34

The number is rather small, but the results evidently agree with expectation.

328 *Spotting in Mice and their Genetic Behaviour*

If heterozygous selfs are mated with the "Daruma," four forms of young should be born in an equal proportion. The results thus obtained are indicated as follows:

		"Kasuri"	"Daruma"	Self	Piebald	Totals
Observed	...	58	63	77	56	254
Expected	...	63·5	63·5	63·5	63·5	254

Inspecting the data above represented it will be seen that the expectation covers almost the actual results, though the self young appear rather numerously.

Mating: "Kasuri" × "Daruma."

If both parents carry the **D** factor in the heterozygous condition we should expect one-fourth of the young to be homozygous **DD** mice. But if such young perish in an early stage of ontogeny, as was shown in the cross "Daruma" × "Daruma," the expected ratio would be modified into a 2:2:1:1 of "Kasuri," "Daruma," self, and piebald respectively instead of a 3:1:3:1, as one of the parents ("Kasuri") carries the **S** factor in a single dose and another one has invariably the composition of **S'S'**. The actual numbers obtained from the reciprocal crosses of this sort are represented in the following table:

		"Kasuri"	"Daruma"	Self	Piebald	Totals
Observed	...	46	39	19	21	125
Expected	...	41·67	41·67	20·83	20·83	125

The results accord nearly with the expectation based on a ratio 2:2:1:1. Thus it reveals the occurrence of the mortality of the **DD** carrying embryos in this cross.

Mating: "Kasuri" × "Kasuri."

When doubly heterozygous "Kasuri" are mated together, the expected ratio is six "Kasuri," two "Daruma," three selfs and one piebald, as young homozygous for the **D** factor will have perished before birth. The results obtained from such a sort of cross are shown in the following table:

		"Kasuri"	"Daruma"	Self	Piebald	Totals
Observed	...	29	9	15	8	61
Expected	...	30·48	10·16	15·24	5·08	61

In this cross, as the factors dealt with are transmitted independently of each other, four sorts of gametes are produced in equal numbers, namely, **1SD:1S'D:1Sd:1S'd**. These gametes would give nine sorts

of zygotes in respect to the genotype and four kinds as regards the phenotype. But actually, subsequent death of the embryos homozygous for **D** would take place, so the ratio which is recognizable after birth of the young is modified, as is represented in the following table:

Race	Genetic composition	Original ratio	Observed ratio
"Kasuri"	SS DD	1	0
"	SS'DD	2	0
"	SS Dd	2	2
"	SS'Dd	4	4
"Daruma"	S'S'DD	1	0
"	S'S'Dd	2	2
Self ...	SS dd	1	1
" ...	SS'dd	2	2
Piebald ...	S'S'dd	1	1

On such an expectation the data obtained can be well understood.

The Behaviour of the D Factor.

Here an attempt will be made to show the total data on the inheritance of the **D** factor, neglecting the other factors and their relation.

In the next table there are summarized the results obtained from the cross **Dd** × **dd** and its reciprocal:

	D	d	Totals
Observed ...	893	981	1874
Expected ...	937	937	1874

The data above represented agree passably with expectation, though the numbers show some marked deviation. But this discrepancy of the ratio is mainly due to the result of the cross **SS'Dd** × **S'S'dd**; omitting this the numbers are 706 **D** and 745 **d**, where expected 725.5 **D** and 725.5 **d** respectively.

The results obtained from the cross **Dd** × **Dd** are summarized and represented below:

	D	d	Totals
Observed ...	284	144	428
Expected ...	285.33	142.67	428

An inspection of the above data shows that the expectation based on a ratio 2:1 covers the results quite fairly. On the normal Mendelian expectation one-third of the **D** factor bearing animals thus obtained should be homozygous as regards this factor. The results, so far as the writers' experiments went, showed that none of them contains the factor in full doses. In all, twelve "Kasuri" (4 ♀♀ and 8 ♂♂) and six

330 *Spotting in Mice and their Genetic Behaviour*

"Daruma" (1 ♀ and 5 ♂♂) coming from the cross "Kasuri" × "Daruma" and its reciprocal, and also six "Kasuri" (1 ♀ and 5 ♂♂) coming from the cross "Kasuri" × "Kasuri," were tested for this purpose, but it was found none of them were homozygous as to the **D** factor. If these numbers are added to those obtained in the case of "Daruma" × "Daruma," the total is fifty-four in number. The evidence strongly supports the view that the mice homozygous for **D** invariably perish at the embryological stage. On this assumption the consequence of the modified ratio 2:1 of **D**:**d**, which resulted from the cross **Dd** × **Dd**, can be clearly understood. The case is similar to that of the yellow mice.

Both the factor for the yellow coat-colour and the **D** factor have a dominant effect upon the somatic character, but act recessively on the lethal result. Analogous cases have been reported by Bridges (1916), Metz (1916) and others in *Drosophila*, and Vilmorin (1914) in *Triticum*. Apart from mice no other similar case has been described in rodents.

The "English" pattern in rabbits is, however, characterized by fine silvered markings of spotting like that of the "Kasuri" race in mice. According to Castle and Hadley (1915) the homozygous animals of this race are quite capable of existence, but have a slightly spotted coat or, as they called them, "Whiter-than-standard." So the factor for the "English" pattern has no influence upon the viability of the animal, even when it is doubled, though the pattern development of this race and the epistatic nature of the factor for the "English" upon the self resemble those in mice.

In studying rats the writers once bred a number of the dark-eyed ones, including, besides pure whites, some slightly spotted ones in a fashion similar to the variation among the "Daruma" race in mice. Unfortunately, however, this experiment had to be abandoned before data sufficient to report were in hand.

In guinea-pigs there are dark-eyed whites sometimes found. According to Castle (1905) the genetic behaviour of this type seems to be complex.

Extracted Self and Piebald.

Extracted selfs and piebalds from crosses where **D** occurs transmit their characteristics by the usual ways as follows: If the homozygous selfs extracted are crossed with pure piebalds the resulting young are all selfs. By two matings of this sort, six animals having self-coloured coat were born. When the heterozygous extracted selfs, however, are mated to piebalds, the two sorts of young result in almost

equal numbers. From eight matings of this sort eighteen self and twenty-two piebald were counted. But if the extracted piebalds are mated between themselves or crossed to piebald of the pure race, they always breed true to the type. By twenty-eight matings one hundred and sixty animals were born and they were all characterized with spotted coat.

Thus the extracted mice of these races having non-contamination of the **D** factor always transmitted their characteristics in the proper manner.

SUMMARY.

1. The results reported in this paper agree in the main with those of Little and Detlefsen. More experimental evidence, however, is supplied by the present investigation.

2. In the present studies, however, the dominant spotting of the "Kasuri" race was distinguished and separately recorded from the recessive ordinary spotting, so the comparison between observation and expectation was more closely dealt with.

3. The **D** factor acting upon **S**, the factor for self, in somewhat restricted fashion on the development of the coat-colour results in the "Kasuri" pattern, and the same factor working upon **S'**, a factor for the spotting, results in a dark-eyed white of the "Daruma" type.

4. On the one hand the **D** factor acts as dominant upon the pattern development as was stated above, and on the other hand it works recessively in regard to the lethal effect. Mice homozygous for this factor perish in the early ontogeny whether they have **S** or **S'** as the hypostatic factors.

5. The "Kasuri" race often become sterile, especially on the female side. This condition was also found to occur in the "Daruma" race in a few instances.

Agricultural College,
Tôkyô Imperial University,
November, 1918.

P.S. This paper had been written some time ago but not published when, quite recently, Little's paper on "The Fate of Individuals Homozygous for Certain Color Factors in Mice" appeared in *The American Naturalist*, Vol. 53, No. 625, 1919.

332 *Spotting in Mice and their Genetic Behaviour*

In that paper he dealt with both the yellow and the "Daruma" races. From his anatomical figures his facts seem to actually break down the homozygous "Daruma" embryo in the early stage of ontogeny like that of the homozygous yellow mice. But this was not completely proven in his article.

EXPLANATION OF PLATE XII.

- Fig. 1. An adult mouse having self-coloured coat.
 Fig. 2. An adult mouse having spotted coat of recessive form.
 Fig. 3. An adult mouse of the "Daruma" race having dark spotted coat. It cannot be distinguished from the lightest spotted form of the "Kasuri" race as represented in Fig. 7, but revealed itself as having genetic composition of $S'S'Dd$ by breeding test.
 Fig. 4. The common types of the "Daruma" race. From the left to the right they represent, respectively, a pure white coated, a white coated having small spots around the ears, and a white coated having small spots in front of the tail.
 Figs. 5 and 6. Two adult "Kasuri" mice having genetic composition of $SSDd$, one relatively light spotted, and another relatively dark spotted.
 Figs. 7, 8, 9 and 10. A series of intensity of spotting in the "Kasuri" race having genetic composition of $SS'Dd$.
 Fig. 11. A litter born in the mating ♀ $S'S'dd \times \text{♂ } SS'Dd$. From top to bottom they represent, respectively, a "Daruma," ditto, a "Kasuri," a spotted, a self, ditto.

LITERATURE CITED.

- BRIDGES, C. B. 1916. "Non-disjunction as proof of the chromosome theory of heredity." *Genetics*, Vol. I.
 CASTLE, W. E. 1905. "Heredity of coat characters in guinea-pigs and rabbits." Carnegie Institution of Washington, Publication No. 23.
 — and LITTLE, C. C. 1910. "On a modified ratio among yellow mice." *Science*, N. S. Vol. xxxii.
 — and HADLEY, PHILIP B. 1915. "The English rabbit and the question of Mendelian unit-character constancy." *Amer. Nat.* Vol. XLIX.
 CUÉNOT, L. 1905. "Les races pures et leurs combinaisons chez les souris (4^{me} note), *Arch. Zool. exp. et gén.*, 4^{me} Serie, T. III., Notes et Revue.
 DETLEFSEN, J. A. 1916. "Pink-eyed white mice carrying the color factor." *Amer. Nat.* Vol. L.
 DUN, L. C. 1916. "The genetic behavior of mice of the color varieties 'Black-and-Tan' and 'Red'." *Amer. Nat.* Vol. L.
 DURHAM, F. M. 1908. "A preliminary account of the inheritance of coat-colour in mice." *Rep. Evol. Comm.* Rep. IV.
 — 1911. "Further experiments on the inheritance of coat colour in mice." *Journal of Genetics*, Vol. I.





Fig. 1.



Fig. 2.



Fig. 3.



Fig. 7.



Fig. 8.



Fig. 9.



Fig. 4.



Fig. 5.



Fig. 6.

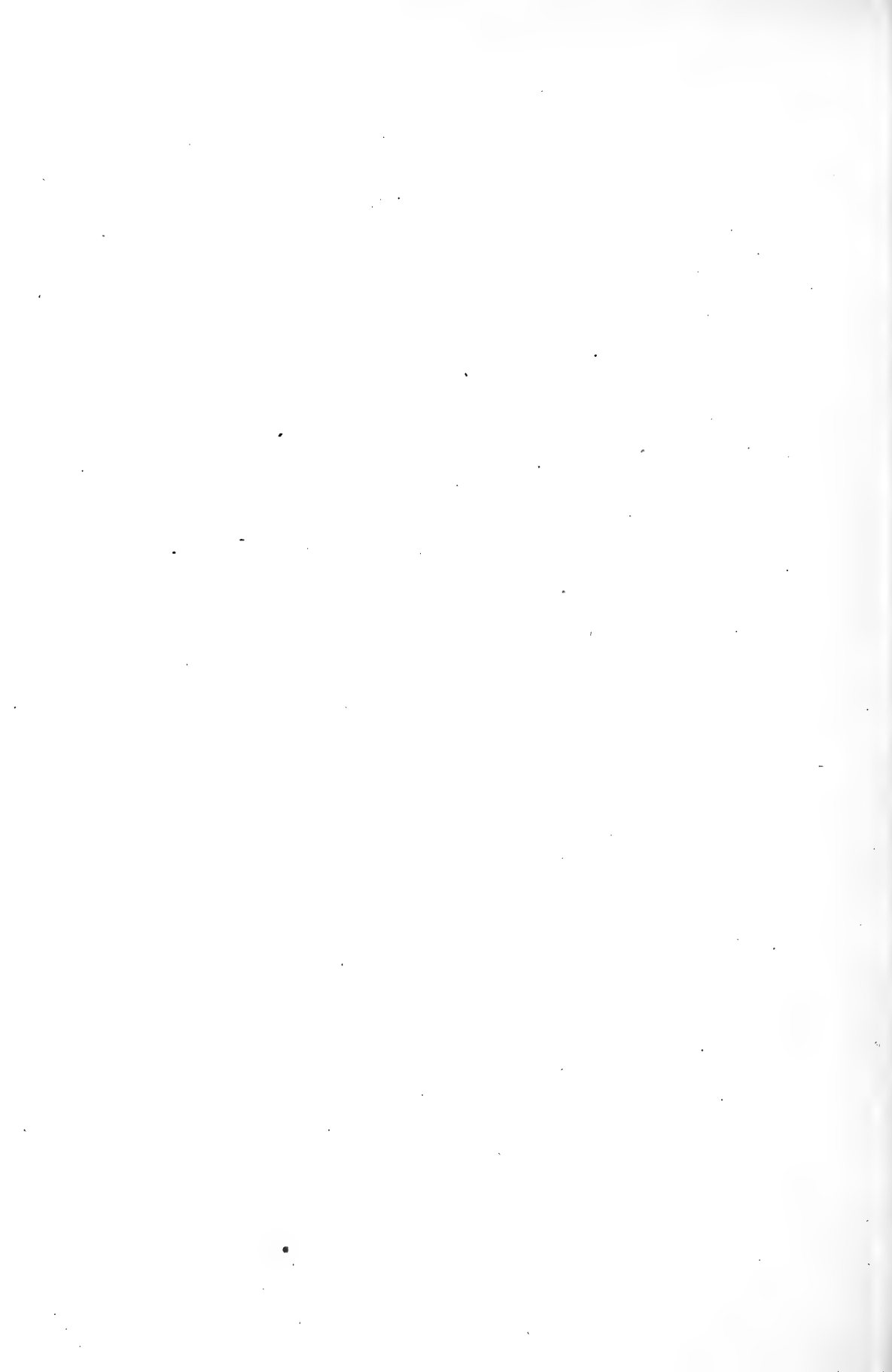


Fig. 10.



Fig. 11.

- HAGEDOORN, A. L. 1912. "The genetic factors in the development of the house-mouse, which influence the coat colour." *Zeitschr. f. ind. Abst. u. Vererb.* Bd. VI.
- IBSEN, H. L. and STEIGLEDER, E. 1917. "Evidence for the death in utero of the homozygous yellow mouse." *Amer. Nat.* Vol. LI.
- KIRKHAM, W. B. 1917. "Embryology of the yellow mouse." *Proceedings of the Amer. Soc. of Zoologists, Abstract. The Anatomical Record*, Vol. XI.
- LITTLE, C. C. 1911. "The 'dilute' forms of yellow mice." *Science*, N. S. Vol. XXXIII.
- 1915. "The inheritance of black-eyed white spotting in mice." *Amer. Nat.* Vol. XLIX.
- 1917. "The relation of yellow coat colour and black-eyed white spotting of mice in inheritance." *Genetics*, Vol. II.
- METZ, C. W. 1916. "Mutations in three species of *Drosophila*." *Genetics*, Vol. I.
- MORGAN, T. H. 1909. "Recent experiments on the inheritance of coat color in mice." *Amer. Nat.* Vol. XLIII.
- VILMORIN, PHILIPPE DE. 1914. "Sur une race de blé nain infixable." *Journal of Genetics*, Vol. III.



THE TORTOISESHELL TOMCAT—A SUGGESTION.

BY L. DONCASTER, F.R.S.

Professor of Zoology, Liverpool University.

IN the *Journal of Genetics*, VIII. 1919, p. 279, C. C. Little puts forward a new hypothesis to account for the origin of the tortoiseshell tomcat. He suggests that the sterile tortoiseshell male is comparable with the *XO* males produced in *Drosophila* by the fertilization of a non-disjunctional ovum bearing no sex-chromosome by an *X*-bearing spermatozoon. Such males in *Drosophila* are sterile, and not infrequently show mosaic characters. Little therefore suggests that the sterile tortoiseshell male is of similar origin, is sterile from the same cause, and is tortoiseshell instead of yellow owing to a tendency to mosaic distribution of the yellow factor. Fertile tortoiseshell males are explained on the assumption that by secondary non-disjunction the *X* and *Y* (*X* and θ) chromosomes come into the zygote from the same parent, and it is suggested that under these circumstances the " θ " chromosome does not have its normal effect, and that what would otherwise have been a yellow male becomes a tortoiseshell. For this second assumption there is little or no real evidence, and its author himself seems somewhat diffident about it. The comparison of the sterile tortoiseshell male with the sterile *XO* type of male in *Drosophila* is at first sight more attractive. The comparison, however, really rests only on the fact of sterility, for the tendency to a mosaic distribution of factors does not seem to be comparable in the two cases. In *Drosophila*, as Morgan, Bridges and Sturtevant show in their recent monograph on *Drosophila* (7), the mosaic distribution of factors is almost certainly due to abnormal distribution of the *X*-chromosome in the segmentation of the egg, and the flies are almost always mosaics of sex-characters (gynandromorphs) no less than of inherited factors. The inherited factors accompany the abnormal distribution of the sex-factors, so that different parts of the fly show not only different inherited characters but also different sexual

features. There seems to be no reason for supposing that this is so, or for expecting that it should be so, in a tortoiseshell cat of chromosome constitution XO instead of XY ($X-$ instead of $X\theta$).

The purpose of this note is to put forward another suggestion—equally speculative, but more easily capable of verification. In a recent paper Magnusson (6) has described the anatomy, both gross and microscopic, of some 70 free-martins. He finds that although in all of them the external features are predominantly female, internally they approximate more or less nearly to the male. In more than half of them the gonads were in the position of ovaries, the uterus was distinctly developed and the vasa deferentia rudimentary, but even in these the gonad was at least as much like a rudimentary testis in structure as like an ovary. The examples described by Miss Chapin (1) from Lillie's material seem to correspond with this group of Magnusson's cases. In the remainder of his cases Magnusson found a series of stages in which the uterus was more and more reduced down to complete absence, the vasa deferentia and epididymis well developed, and in several instances the gonads had passed into the inguinal canal. In these examples of more male type the testis contained seminal tubules with interstitial tissue, including Leydig's interstitial cells, between them. The tubules sometimes contained Sertoli cells, but never any trace of true seminal cells, and Magnusson describes them as closely similar to those found in retained (cryptorchid) testes of true males. Further, it should be noted that in a number of his examples the interstitial tissue was much more abundant than in normal testes. Magnusson's description of the microscopic structure of the gonad in the more masculine of his free-martins in Cattle immediately recalls the condition described by D. W. Cutler and the writer (2) in the testis of a sterile tortoiseshell tomcat; the structure of the cat's testis appears to be almost identical with that of the gonad in some of the free-martins. This suggests the possibility that the tortoiseshell tomcat may possibly be in fact a free-martin. Lillie (4) has shown almost beyond doubt that the free-martin is derived from a female embryo which has been "masculinized" by the confluence of its vascular system with that of a neighbouring male foetus. Magnusson, not knowing Lillie's work, believes that the free-martin is one of a pair of uniovular male twins, but his evidence for this is entirely unconvincing, and it may be assumed with confidence that Lillie's explanation is the true one. If in Cats, as in Cattle, the embryonic membranes of two foetuses may coalesce so that their blood-system becomes confluent, and if in them the same masculinization of a female embryo results,

occasionally a tortoiseshell female embryo would be masculized. It must be assumed that the masculization is more complete in the Cat than in Cattle, so that the testes, instead of only comparatively rarely descending into the inguinal canal, regularly reach the scrotum, and the external genitalia also assume the male type. Since, however, Magnusson finds a series of stages between an almost female and a nearly male condition, it is not a very improbable assumption that in the cat the process is habitually carried further. Possibly the fertile tortoiseshell male, which on the evidence of Sir Claud Alexander (quoted by Cutler and Doncaster) must be admitted to exist, is the final stage of the series. Complete conversion into males of embryos which are genetically female appears to exist in some of Goldschmidt's *Lymantria* crosses (3) and in several hybrids between different species of moths.

This hypothesis involves the assumption that females of colours other than tortoiseshell must also at times be changed into apparent males, but since these would naturally be regarded as males which for some reason or other were not successful breeders, no record of them would be preserved. The suggestion here put forward does not seem difficult to test. By collecting gravid uteri of cats and examining the foetal membranes it should not be difficult to find out whether confluence between the blood-systems of neighbouring foetuses ever takes place. If it did, and if one of the foetuses was definitely male, then according to the hypothesis the other foetus should, in two cases out of three, have testis-like gonads with no seminal cells in the tubules.

The writer does not feel confident of being able to carry out the considerable labour involved in testing the hypothesis in the immediate future, and he puts it forward in the hope that some other may be able to obtain and examine the necessary material.

PAPERS REFERRED TO.

1. CHAPIN, CATHARINE L. "A microscopic study of the reproductive system of foetal free-martins." *Journ. Exp. Zool.* Vol. XXIII. 1917, p. 453.
2. CUTLER, D. W. and DONCASTER, L. "The Sterility of the Tortoiseshell Tom-cat." *Journal of Genetics*, Vol. v. 1915, p. 65.
3. GOLDSCHMIDT, R. "Experimental Intersexuality and the Sex Problem." *Amer. Nat.* Vol. L. 1916, p. 705.
4. LILLIE, F. R. "The Free-martin: a study of the action of sex-hormones in the foetal life of cattle." *Journ. Exp. Zool.* Vol. XXIII. 1917, p. 371.

5. LITTLE, C. C. "Colour Inheritance in Cats, with special reference to the Colours Black, Yellow and Tortoiseshell." *Journal of Genetics*, Vol. VIII. 1919, p. 279.
6. MAGNUSSON, H. "Geschlechtslose Zwillinge. Eine gewöhnliche Form von Hermaphroditismus beim Rinde." *Arch. f. Anat. u. Physiol. Anat. Abt.* 1918, Vol. I. p. 29.
7. MORGAN, T. H. and BRIDGES, C. B. "The Origin of Gynandromorphs." In "Contributions to the Genetics of *Drosophila melanogaster*." *Carnegie Inst. Publ.* Washington, 1919.

THE INHERITANCE OF WING COLOUR IN LEPIDOPTERA.

III. *MELANISM IN BOARMIA CONSORTARIA* (VAR. *CONSOBRINARIA*, BKH.).

By H. ONSLOW.

(With Plate XIII.)

THE inheritance of melanism in *Tephrosia consonaria*¹ was described in a recent communication, and further experiments with a similar form of *Boarmia abietaria* will appear shortly. The work here detailed was carried out with the melanic form of *B. consortaria* (the Pale Oak Beauty). The three melanic varieties just mentioned are peculiar, because their origin is confined to the South of England. Black specimens of *T. consonaria*, as well as those of *B. consortaria* first appeared in the same oak-wood in Kent, and were not found far from that neighbourhood until recently. Also the melanic form of *B. abietaria* occurs only in Surrey, and occasionally in the New Forest. In none of these three species did the melanism originate in the industrial districts of the North. Lately, a melanic form of *T. extersaria* has arisen in Kent.

In all accounts of the progressive spread of melanism, great stress is laid on the fact that the melanic varieties have been found chiefly in the "Black Country" of England, and in a similar district round Crefeld in Germany. The deposit of soot and chemicals on the trees etc. in these districts is supposed to give to the melanic varieties a survival value, on account of their colouring, which there becomes protective. There seem to be grave objections to this theory. In the first place some typically melanic varieties have arisen in the rural districts of the South (as is the case with the three species just mentioned), and other varieties are found in equally rural parts of the North and West. Secondly, most melanic moths are night flying, only occasionally being seen sitting on trees,

¹ Onslow, H., *Journal of Genetics*, Vol. ix. No. 1, p. 53, Dec. 1919.

palings, etc. in the daylight; and at night all moths are melanic. It is true that a species like *Gnophos obscurata* has a white form confined to the chalk district of Lewes, and a dark form common in the New Forest, where the insect is said to rest on cut peat; but the contrast between the colour of chalk cliffs and that of peat hags is much greater than between urban and rural districts. Geological formations, moreover, have been able to exert their influence for a much greater period than the works of man. It would be dangerous to say that the black colour of the melanic forms has no protective value, but it is hardly credible that it can be the chief, or only factor in their rapid appearance and increase.

It might, at first sight appear that if a new form is dominant, as is the case in most melanic varieties, nothing else would be required to ensure that it gradually replaced the type form. It has however been shown on mathematical grounds¹, that if a dominant character is introduced into a normal population, in which mating takes place at random, the proportion of this form in the second generation will be double what it was in the preceding one, but this proportion will afterwards have no tendency whatever to increase. Some other factor which favours the variety at the expense of the type form must therefore be postulated.

It appears much more reasonable to suppose that the black colour has little or nothing to do with the matter, but that the real cause lies in a constitutional hardiness, which is correlated with melanism. The black colour is such a striking feature that far too great an effect is likely to be attributed to it. Doncaster², who first made this suggestion, has observed that the banded variety of *Angerona prunaria*, var. *sordidata*, is less hardy than the type, and Bowater³ states that the melanic variety of *Odontoptera bidentata* is also the hardier. In the experiments here recorded, as well as in those dealing with *T. consonaria*, var. *nigra*, and other melanic forms, the general impression is that the melanics seem to be earlier, stronger and larger than the type forms. Moreover, there is often a slight excess of melanics in most crosses.

Some explanation is certainly necessary to account for the fact that melanic varieties occur with such frequency in urban and industrial districts. If however it be supposed that many species have "sporting," giving melanic varieties, which are hardier and more robust than the type insects, it follows that these melanics will increase rapidly at the expense of the feebler form, wherever the struggle for existence is

¹ Hardy, G. H., *Science*, N. Y., Vol. xxviii. N. S. No. 706, p. 49, July 10, 1908.

² Doncaster, L., *Ent. Rec.* Vol. xviii. p. 219, 1906.

³ Bowater, W., *Journal of Genetics*, Vol. iii. p. 299, 1914.

severest. These conditions could not be better fulfilled than in the manufacturing areas, where the woods and vegetation upon which the larvae feed have been largely destroyed and elsewhere contaminated with a chemical deposit.

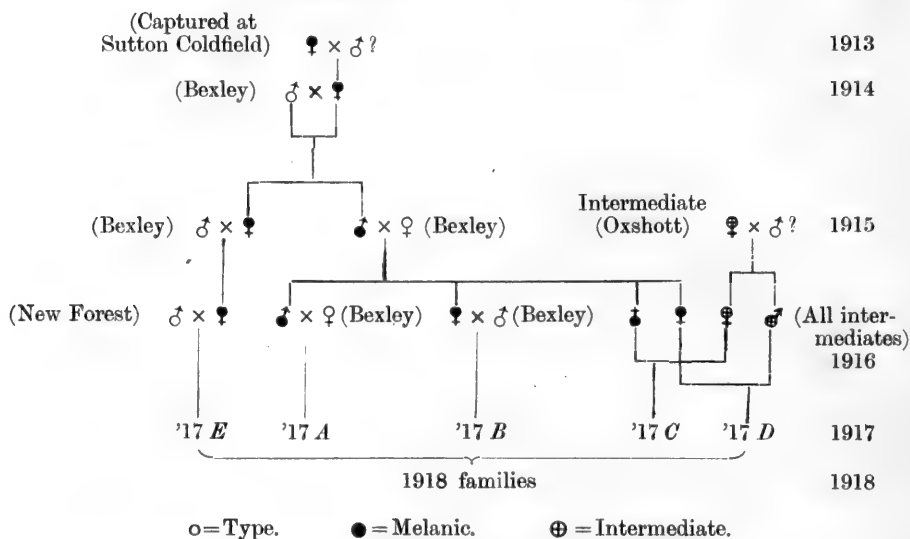
E. Goodwin originally found the melanic form of *B. consortaria* in the same oakwood in North Kent in which he took the black form of *T. consonaria*. There is a mention of the Kentish form by R. South¹, which is referred to var. *humperti*, Humpert. The second line of the forewings is, however, edged with white, and there is a white sub-marginal line in the English form, but both markings are lacking in var. *humperti*. All the melanic specimens bred in these experiments had the white lines more or less well developed. E. A. Cockayne reports taking a typical ♀ at Oxshott in 1914, which had evidently paired with a melanic ♂, because the ova deposited produced about equal numbers of both forms. A virgin melanic ♀ was taken at Oxshott by the same observer in 1919. These melanics he refers to var. *consobrinaria* Bkh. There are at least two other references to captures at Oxshott, where a large proportion of melanics are said to occur every year, and another specimen was taken at Chislehurst, so that it seems to have soon spread. Mr L. W. Newman tells me that in 1916 he received a dark ♀ captured at Oxshott, which in the following year produced a number of specimens, all dark like their parent, and yet considerably lighter than the melanic form. It appears therefore that either the melanic specimens at Oxshott vary considerably, or else an intermediate form must exist. Two of these dark Oxshott insects are shown in Plate XIII, Figs. 3 and 9.

The original melanic stock which supplied material for these experiments was a melanic ♀ from Mr Newman captured at Sutton Coldfield in 1914. This appears to be the first record in Britain of a specimen of the melanic variety being taken outside Surrey. Though all the Surrey specimens may have spread from the original mutant in the Kentish oakwood, it is hardly likely that a specimen could have reached Warwickshire, unless by the aid of man, so it seems legitimate to suppose that this race must have originated *de novo*; this time, be it observed, in the close proximity of Birmingham. The pedigree on the next page gives the parentage of the families dealt with in this paper.

It will be seen that Mr Newman paired two of the intermediate Oxshott insects with two melanic specimens of the Sutton Coldfield strain, and two of the latter strain were paired to type insects. Three of the resulting families are shown on p. 344 (i.e. '17 A', '17 B', '17 D').

¹ South, R., *Moths of the British Isles* (F. Warne, 1909).

GENEALOGICAL TABLE.

B. consortaria and var. *consobrinaria*.

Family '17 C came from a pairing of an intermediate ♀ (Oxshott) × melanic ♂ (Sutton Coldfield). Instead of producing the expected 50 per cent. melanic, it gave only 4 melanics and 63 type. As this result is quite unlike any of the later broods obtained, it has not been entered in the tables, on account of the possibility of confusion before the ova were sent. Moreover, I have not seen the parents, and there is always the chance that the ♂ parent may have been a dark intermediate and not a melanic. It should be mentioned that Mr Newman reported getting 4 per cent. melanics from pairings between the original melanics and type insects.

It is quite possible that some of the specimens in '17 C and '17 D may have been intermediate like one of their parents, but as they were most of them used for breeding, the specimens were rubbed, and it was impossible to judge them accurately. One of the 1918 families, however, '18 L, instead of producing equal numbers of melanics and types, as was expected, gave melanics and a distinct intermediate form, which was a peculiar dark brown, quite unlike all other specimens. This form is illustrated in Plate XIII, Figs. 4 and 10. Comparison between Figs. 3 and 9 and Figs. 4 and 10 shows the latter to be not unlike the dark variety from Oxshott, though slightly paler, forming as it were a link

with the types. The type specimens Figs. 1, 2, 7 and 8 all vary appreciably. The females are certainly greyer than the males, many of which have a pale buff line giving the whole insect a slightly yellow appearance. The darkest type specimen bred is shown in Fig. 8. There were only a few insects as dark as this, but there is always the possibility that they may have been specimens of the dark intermediate form. A number of pairings were carried out with the 1917 families, crosses being made with the melanic strain and a race of type insects from pupae collected in the New Forest. The average appearance of these insects, one of which is illustrated in Fig. 2, is much the same as that of the types extracted from the melanics, a typical specimen of which is shown in Fig. 7. The melanics were all of a deep grey with a white sub-marginal line, as shown in Figs. 5, 6, 11 and 12. There was some variation, certain insects being even paler than Fig. 11, but they were all without exception quite distinct from both type and intermediate specimens, chiefly owing to the lack of any pattern, even in the palest insects. This difference, though very distinct soon after emergence, when all records were made, gets much fainter after the insects have been dried some months.

The larvae proved very hardy, especially the melanic race. They were fed entirely with oak, upon the hard, older leaves of which they thrived remarkably well. They were kept in the usual glass breeding cylinders, and in the spring of 1919 over a thousand insects emerged.

The result of mating together melanics, both of which were evidently heterozygous, was as follows :

Melanic \times *Melanic*.

DR \times *DR*.

Family	Imagines					
	Melanic			Type		
	Male	Female	Totals	Male	Female	Totals
'18 <i>G</i>	19	15	34	12	10	22
'18 <i>H</i>	3	12	15	3	5	8
'18 <i>I</i>	10	11	21	2	2	4
'18 <i>J</i>	23	27	50	13	8	21
Totals	...		120 (69%)			55 (31%)
Expectation			131.25			43.75

Unfortunately, no matings could be made with homozygous melanics¹,

¹ Through the courtesy of Prof. J. W. H. Harrison, who has bred this species extensively, I am enabled to say that in 14 broods of melanic by melanic, he obtained 662 melanic ♀♀ and 653 melanic ♂♂, but no types.

344 *The Inheritance of Wing Colour in Lepidoptera*

as all the melanics in the previous year had been paired to types. Several matings of this description have however now been made, and the results will be known next summer. The expected ratio of 3:1 is not very closely approached in the above table, there being an excess of nearly 6 per cent. of types, which is unusual. The numbers are however not very large, but clearly the divergence is due principally to family '18 *G*, which from the ratio of the offspring might almost belong to the *DR* × *RR* type of mating.

The result of mating heterozygous melanics by type was as follows :

Melanic × *Type*.

DR × *RR*.

Family	Bred by	Female	×	Male	Imagines					
					Melanic			Type		
					Male	Female	Totals	Male	Female	Totals
1914	E. Cockayne	Type (Oxshott)	×	♂?	18	25	43	21	19	40
'17 <i>A</i>	Ova from	Type	×	Melanic	1	7	8	14	8	22
'17 <i>B</i>	L. Newman	(Bexley)	×	(Sutton Coldfield)	12	5	17	4	8	12
	"	Melanic (Sutton Coldfield)	×	Type (Bexley)						
17 <i>D</i>	"	Melanic (Sutton Coldfield)	×	Dark Intermediate (Oxshott)	14	14	28	10	3	13
'17 <i>E</i>	H. O.	Melanic (Sutton Coldfield)	×	Type (New Forest)	10	8	18	11	14	25
'18 <i>Z</i> *	H. O.	?	×	?	11	18	29	11	9	20
'18 <i>B</i>	H. O.	Type (ex '17 <i>C</i>)	×	Melanic	7	5	12	7	3	10
'18 <i>C</i>	H. O.	Type (ex '17 <i>A</i>)	×	Melanic	4	10	14	4	9	13
'18 <i>D</i>	H. O.	Type (ex '17 <i>C</i>)	×	Melanic	49	64	113	60	49	109
'18 <i>E</i>	H. O.	Type (ex '17 <i>C</i>)	×	Melanic	5	3	8	5	2	7
'18 <i>F</i>	H. O.	Melanic	×	Type (ex '17 <i>E</i>)	24	22	46	23	25	48
'18 <i>M</i>	H. O.	Type (New Forest)	×	Melanic	4	6	10	9	6	15
'18 <i>N</i>	H. O.	Melanic	×	Type (New Forest)	16	18	34	18	13	31
'18 <i>L</i>	H. O.	Melanic	×	Type (New Forest)	35	27	62	43	17	60
Totals					442 (51%)			425 (49%)		
Expectation					433.5			433.5		

* The ova from this pairing were given to Mr W. Moore, who very kindly reared them for me and showed me the resulting specimens. The identity of the parents, however, has unfortunately been lost.

The melanics were all bred from one or other of the 1917 melanic × type or melanic × intermediate pairings, shown in the pedigree. In

five cases the type parents come from 1917 pairings of melanic \times type, which are indicated in brackets, and in all other cases they come from wild pupae. The expected ratio of half and half is seen to be approached very closely, and there is probably little doubt that the previous table would not have shown such a wide divergence, had the numbers been as considerable. The last family in the table, '18 *L*, the numbers of which have been printed in italics, produced, not melanics and types, but melanics and intermediates (see Plate XIII, Figs. 4 and 10) in equal numbers. As has been said, this dark form, though slightly darker than the Oxshott strain, was probably introduced by it, since the melanic ♀ parent came from (Oxshott) ♀ \times (Sutton Coldfield) ♂. A number of further matings have, however, been carried out with this form, and the results, if they prove of any interest, will be published in due course.

Attention should perhaps be called to the curious inverse relationship between sex and colour in some of the above matings, there being an excess of one sex in the melanic offspring, and an excess of the other sex in the type offspring. This occurs in families 1914, '17 *A*, '17 *B*, '17 *E*, '18 *D* and '18 *M*, there being as a rule an excess of melanic ♀♀ and of type ♂♂ when the ♀ parent is a type, and an excess of melanic ♂♂ and type ♀♀ when the ♀ parent is melanic. It is quite probable that this relationship may be fortuitous, as it does not occur in nearly all the families. Nevertheless, '17 *A*, '17 *B*, and '17 *E* are all descended from the same strain of melanic parents, and in both the families '18 *M* and '18 *D*, the melanic parent comes from '17 *E*.

A small number of type insects were also paired together, and as was expected, they all gave nothing but types.

Type \times *Type*.

RR \times *RR*.

Family	Bred by	Female \times	Male	Imagines					
				Melanic			Type		
				Male	Female	Totals	Male	Female	Totals
'17 <i>T</i>	Ova from L. Newman	Type \times (Bexley)	♂ ?	—	—	—	8	6	14
'18 <i>A</i>	H. O.	Type \times (ex '17 <i>A</i>)	Type (ex '17 <i>C</i>)	—	—	—	62	43	105
'18 <i>Q</i>	H. O.	Type \times (ex '17 <i>D</i>)	Type (New Forest)	—	—	—	45	23	68
'18 <i>K</i>	H. O.	Type \times (ex '17 <i>E</i>)	Type (ex '17 <i>D</i>)	—	—	—	43	45	88
Totals				—	—	—	—	—	275

The breeding of the above parents may be found from the genealogical table. The ♀ parent of '18 *Q* was selected, as being one of the darkest types, similar to Fig. 8, Plate XIII. Both parents of '18 *K* were the same shade, nevertheless only a few per cent. of the offspring approached the same depth as their parents.

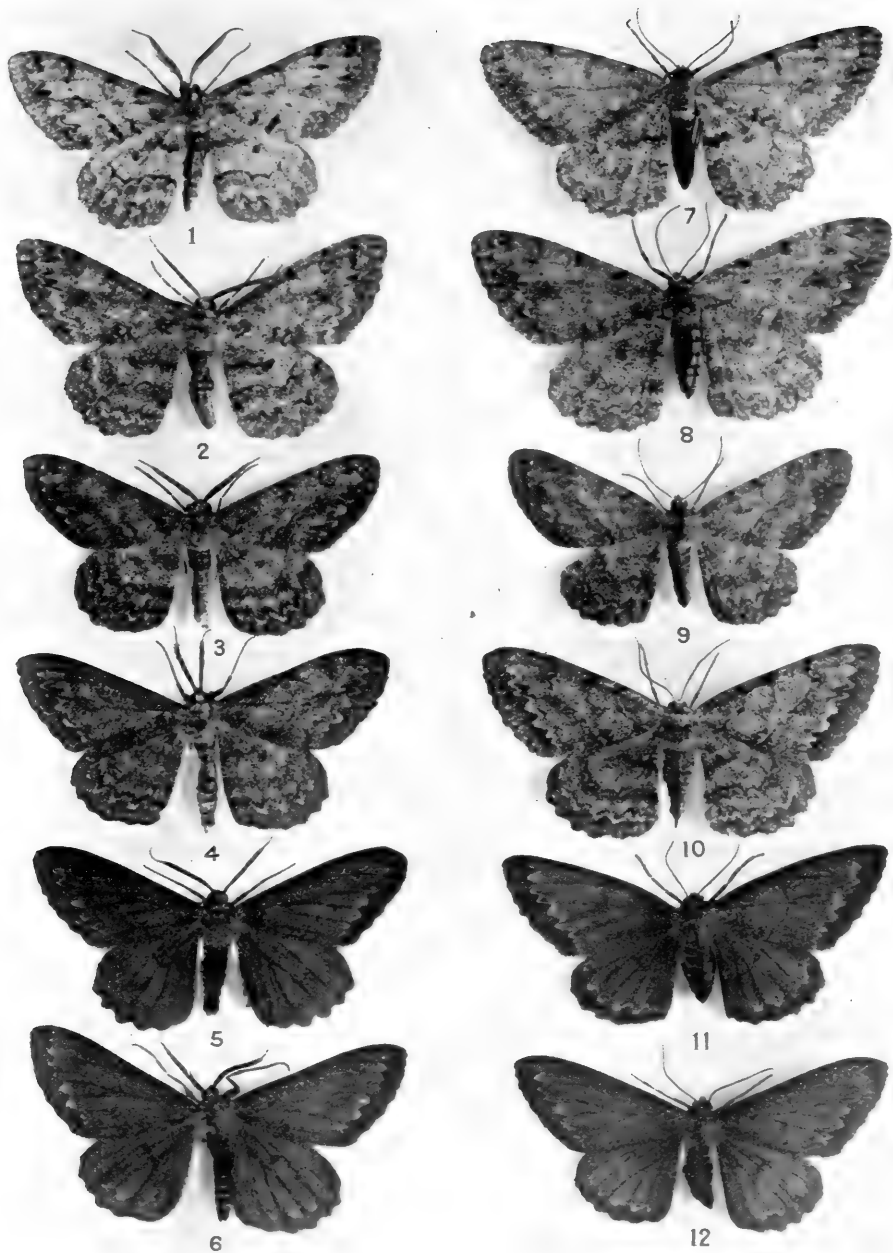
It can be concluded, therefore, that in the case of *B. consortaria*, as in that of *T. consonaria*, the melanic variety is a simple mendelian dominant.

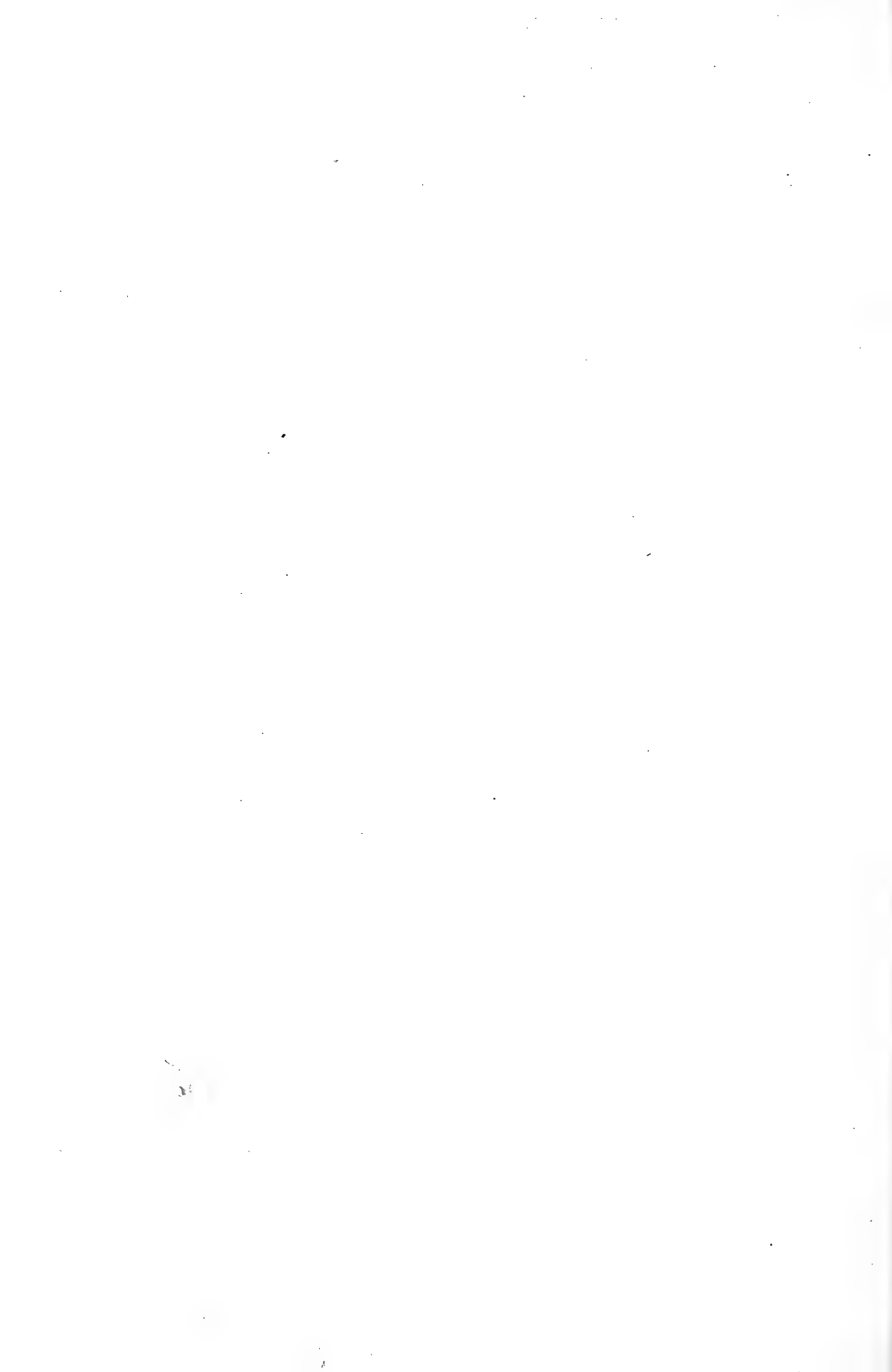
My thanks are due to Mr L. W. Newman for supplying me with my original material. I am also indebted to Professor Harrison, Professor Doncaster and Professor Punnett for help in the preparation of this paper, and to Miss Helen Moodie for her constant care of the larvae.

DESCRIPTION OF PLATE XIII.

Boarmia consortaria and var. *consobrinaria*. Natural size.

1. *B. consortaria* ♂. Type, bred from type × melanic.
2. *B. consortaria* ♂. Type (New Forest).
3. *B. consortaria* ♂. Dark intermediate from Oxshott.
4. *B. consortaria* ♂. Dark brown intermediate from family '18 *L*.
5. Var. *consobrinaria* ♂. Melanic.
6. Var. *consobrinaria* ♂. Melanic.
7. *B. consortaria* ♀. Type, bred from melanic × melanic.
8. *B. consortaria* ♀. Type.
9. *B. consortaria* ♀. Dark intermediate from Oxshott.
10. *B. consortaria* ♀. Dark brown intermediate from family '18 *L*.
11. Var. *consobrinaria* ♀. Melanic.
12. Var. *consobrinaria* ♀. Melanic.





EYE-COLOUR IN GAMMARUS.

By E. J. ALLEN, D.Sc., F.R.S., AND E. W. SEXTON, F.L.S.

From the Laboratory of the Marine Biological Association, Plymouth.

(With Plate XIV and One Diagram.)

CONTENTS.

	PAGE
I. Colour associated with the Perfect Form	347
II. Absence of Colour associated with Imperfect Form	349
III. The No-white Mutation	350
IV. Spots	352
(1) The Appearance of Spots in the Pure Red Stock	354
(2) Appearance of Spots in the Albino Stock	355
(3) Selected Cases	356
(4) Attempts to establish Pure Stocks	358
A. Experiments to establish an Unspotted Strain	358
B. Experiments to establish a Pure Spotted Strain	361
1. Albinos mated with Reds	361
2. Tested Albinos mated with tested Albinos	363
V. Spots with the No-white Mutation	364

THE eyes of *Gammarus chevreuxi* with which we are dealing are of two kinds, derived from the same stock, a Perfect Form with Colour,—the “Normal” eye—and an Imperfect Form, with no colour, which we call the “Albino” eye. (See Allen and Sexton, *Journ. Marine Biol. Assoc.*, Vol. XI, No. 3, Dec., 1917, p. 274.)

I. COLOUR ASSOCIATED WITH THE PERFECT FORM.

In the wild state the eye is reniform in shape, with the margin entire, and consists of a large number of ommatidia arranged in regular rows, the number increasing with each moult from 10 to 12 in the newly hatched to 70 or 80 in the adult. (Plate XIV, Fig. 1.)

Each ommatidium is surrounded by five deeply pigmented retinal cells. The retinal pigment in the wild animal is black, but a mutation occurred in the second generation from adult animals brought into the laboratory. In this mutation the eye-colour was red. In addition to this pigment, there is a superficial layer of chalky-white pigment spread

over the surface of the whole ommateum, the lenses showing as circles of colour enclosed in a white meshwork or reticulation.

The white pigment disappears within an hour or two of death, and is soluble in all the usual preserving fluids.

The black retinal pigment on the other hand is practically permanent; the red lasts for some time in preservatives but eventually fades out.

Mated together, Black proved dominant to Red, giving F_2 in the proportion 3:1, and three stocks were established—Pure Black, Hybrid Black (i.e. Black carrying Red), and Pure Red¹.

Colour is always associated with the Perfect Form. In only one case has an exception been noted. This was in the Pure Red stock where two succeeding broods of Reds each contained one specimen in which the form of the eye was perfect, but the retinal cells were unpigmented. One, a male, survived to mate, and was put with a degenerate-eyed Albino female (see Imperfect Form). The result of this mating was a brood of coloured offspring. Experiments (given in detail in Allen and Sexton, 1917, pp. 287–325) showed the lack of colour to be due to some obscure pathological cause, the animal functioning as a Normal Red-eye.

The same thing, but in a much less degree, has occurred in several of our stocks, animals both Black and Red were found with patches of uncoloured ommatidia in otherwise normal eyes. These were bred together but in all cases the succeeding generations were normal-eyed, proving the condition to be not inheritable.

The stock, from which the two white-eyed specimens described above were derived, was kept under observation until it died out, November, 1918, but beyond a few instances of dilute Red pigment, nothing unusual has been noted.

No second case of the Red-eye mutation has arisen independently up to the present date.

Darkening of Red pigment with age. It must be noted here that the retinal pigment in Red eyes is not always of the normal bright-red tint. It tends to darken with age; occasionally all the ommatidia become so dark as to appear black, in other instances dark ommatidia develop among the red, especially in the middle of the eye. In the case figured, that of the female I.E. (see Plate XIV, Fig. 2, and p. 356) which had the record number of young, 780 in all, the dark pigment increased with age, until the eye looked black. The figure was drawn not long

¹ In this paper we use the term "Pure" in reference to colour simply to denote the absence of the Albino factor. Pure Black, Pure Red, means Black or Red without the Albino factor, "Hybrid" Black means Black carrying Red, but no Albino.

before the female died. Her last broods had eyes of very dilute red, pale pink in some, and pale yellow in others, but these, after moulting, all became bright red.

Adult specimens are sometimes met with, the eyes of which have very dark pigment in the centre and bright red round; sometimes after moulting the eyes are bright red, more rarely they remain unchanged. Only three young have been recorded from the red stock with eyes at birth so dark as to appear black. Two died soon after extrusion, but the third came to maturity, a male, and its eyes were then red with dark centres. It was mated with a female with similar eyes and had numerous offspring all with the normal bright red colour.

II. ABSENCE OF COLOUR ASSOCIATED WITH IMPERFECT FORM.

A second mutation appeared in the F_2 generation from a cross between Pure Black and Pure Red. Out of a brood of 12, 8 had normal eyes, 7 Black and 1 Red, the remaining 4 were what we call the "Albino" eyes (Plate XIV, Fig. 3).

The Imperfect Form differs from the Perfect or Normal in *shape*, which varies with the individual, and even in the same individual; in the *margin*, which is always irregular and ragged in outline, and generally deeply indented and broken up; in the *number* of the ommatidia, which are always few in number, never reaching to even half the number in the normal; in the *size* and *shape* of the lenses of the ommatidia, which are always variable; in the disposition of the *white pigment*, which is not spread evenly between the ommatidia as in the normal, but appears as masses or patches of white with the ommatidia scattered here and there in no sort of regularity, frequently with some lying beyond the margin of the ommateum, quite apart from the pigment.

The principal difference between the two forms lies in the *Absence of Retinal Colour*, and this character is always found associated with the Imperfect shape of the eye.

The Perfect Form with Colour is dominant to the Imperfect Form without Colour (i.e. Albino) giving F_2 in the proportion 3:1.

The Albino eyes all carry the factor for Colour, some the factor for Pure Black, others the factors for Black and Red, and others again for Pure Red, but, unless mated with Colour, their constitution cannot be ascertained. Mated together, they give Albinos.

Only once, in the whole course of the experiments, has an instance occurred in which two degenerate Albino-eyed animals produced a normal

Black-eyed young. This pair (the C. 17*b* referred to on p. 358) had 12 broods, numbering 249 young in all. The first 8 broods were as follows:—15, 30, 22, 7, 22, 37, 30, and 12, all Albinos, and then came a brood of 2 only. The two young were seen moving in the pouch the day before extrusion. When extruded they proved to be one Black perfect-eyed animal and one Albino. The Black one had a spot of white pigment on the right side, on the edge of the brain, the Albino one had eyes of nearly perfect form, but with the ommatidia few in number, and arranged around the margins. Both died before reaching maturity. There was an interval of two months without any young being hatched, and then broods of 25, 42, and 5, all Albinos.

As with the Red-eyed mutation, no second case of Albino eyes arising independently has been seen up to the present time.

III. THE NO-WHITE MUTATION.

The white pigment is subject to great variation. In the coloured eyes it occasionally appears as a very heavy reticulation almost obscuring the ommatidia, and accompanied by a loss of brilliancy in the coloured pigment: or it may be so much reduced in quantity that the reticulation only shows as a thread-like network between the ommatidia: or, as happens in many cases, only streaks or flecks of white are left. When it is entirely lacking, the typical *No-whites* are produced.

These No-whites appear in both the Perfect and Imperfect Forms, the Absence of the white pigment acting as a simple recessive to its Presence (Plate XIV, Fig. 4).

The Albino No-white we call the "Colourless" eye, as it lacks both the coloured and the white pigment (Plate XIV, Fig. 5).

The No-white mutation seems by no means an uncommon one. It has appeared in some of the families in all our stocks of *Gammarus chevreuxi*, and probably occurs in other Amphipod species. Owing to the white pigment disappearing in preservatives, it is not possible to judge from pickled specimens, but one case has been noted in the living ones. Another species of brackish water *Gammarus* inhabits the ditches with *G. chevreuxi*, and one animal was found, in a dredging, with both eyes No-white.

Origin of No-white Mutation. This mutation begins in at least two different ways: (a) it may appear suddenly either with one eye or with both eyes affected; or (b) it may develop gradually, e.g. parents with "thin reticulation" giving some young with thin reticulation, and some

No-white; or, as in an instance referred to later, the young may be hatched with the white pigment apparently normal, and yet suffer a gradual reduction of it until at maturity they are either practically or completely No-white.

Some of the experiments are mentioned below in order to show these distinctions.

(a) The first record of the sudden appearance of a No-white was in November, 1914, in the F_2 descendants of one of the crosses made between the Pure Black and the Pure Red strains, which had been kept under laboratory conditions for two and a half years, since June, 1912.

In all, 5596 F_2 young were hatched during the period September, 1914 to September, 1915, and among these 3 typical No-whites appeared, and 13 one-sided No-whites (i.e. one eye with normal white reticulation and one No-white).

Some similar experiments have been described in a previous paper, Allen and Sexton, 1917, pp. 326-330, and 336-341, which see also for one-sided No-white matings with normal-eyed animals (p. 339).

(b) The following experiment illustrates the gradual development of No-white.

Two animals were taken from the Pure Black normal-eyed stock brought in, February, 1915 (Pair V, see p. 329, Allen and Sexton, 1917, *Journ. M.B.A.*, XI.). The eyes of the male were normal, while those of the female had less white pigment than usual. One of the F_1 generation, a male, had very thin reticulation in its eyes; it was mated with a female of the same brood, one brood of 13 was hatched, and then it ate her—before her eyes had been examined. Of this brood, extruded November 15, 1915, 11 had thin reticulation, and the remaining two were typical No-whites. These were left to interbreed, and examined at intervals. In September, 1917, 12 young were seen, 10 practically normal-eyed, and the two which were separated for this experiment and kept under daily observation.

These were, a male with very little white in the eyes, and a female with the Right eye No-white, and regular but very thin reticulation in the Left eye, and a tiny deep spot on the left side of the head (i.e. three eyes with thin reticulation and one eye No-white).

The experiment gave an unexpected result. 311 young in all were hatched, all except two with perfectly normal eyes, eight of them with more white than usual and 170 "spotted" as well. (The two exceptions each had one eye No-white.) But by the time they reached maturity the white pigment had either vanished altogether or become reduced

in all the survivors to a thin thread-like reticulation. This reduction of the white pigment took place gradually. All those examined when half-grown were found with *very* thin reticulation, whilst at maturity some eyes could not be distinguished from typical No-whites, in others only a fleck or two of white was left; in others one eye had developed into No-white, and one had thin reticulation; in some again the "spots" had disappeared. There was not one single instance in four generations of an animal reaching maturity with the reticulation normal.

The details summarised are as follows:—

In the F_1 generation; 22 young, 10 of them spotted, 1 No-white in Right eye: 11 survivors, all practically No-white.

In the F_2 generation, spotted matings; 88 young normal-eyed, 34 of them spotted: 11 survivors, 2 one-sided No-whites, the others either practically or completely No-white.

In the F_3 generation, unspotted matings; 34 young normal-eyed, 7 of them spotted: 9 survivors, all became completely No-white.

In the F_3 generation, spotted matings; 32 young normal-eyed, 18 spotted: 4 survivors, 1 completely No-white, the others practically so.

In the F_4 generation, unspotted matings; 56 young, 1 of them No-white in Left eye, 1 with very heavy white reticulation in Left eye, the others normal-eyed, 40 of them spotted: 7 survivors, No-white and practically No-white.

The F_2 and F_3 generations were crossed, spotted matings; 47 young of which 41 were spotted, and 4 with rather heavy reticulation: 4 survivors—No-white.

The F_2 generation from these crosses, spotted matings; 38 young, 28 of them spotted, 2 with heavy reticulation.

IV. SPOTS.

In the Albino-eyed stocks used in the experiments the white extra-retinal pigment shows a tendency to break up, and portions of it become detached from the eye. These form definite spots of chalk-white pigment, and these spots do not contain ommatidia (Plate XIV, Fig. 6). What appear to be exactly similar white spots have occurred from time to time in all the experimental stocks (red, black and hybrid), but they seem to run in particular families. They are especially frequent in the Albino stock, and to a less extent in Red carrying Albino. The spots vary greatly in size and shape, being most often small spherical masses, at other times becoming white streaks of varied length and width, and

less frequently large, white irregular patches, which in the Albino stock may be even as large as the eyes in extreme cases. All these different kinds we speak of generally as *spots*. They are found most frequently in certain definite positions as shown in Diagram 1.

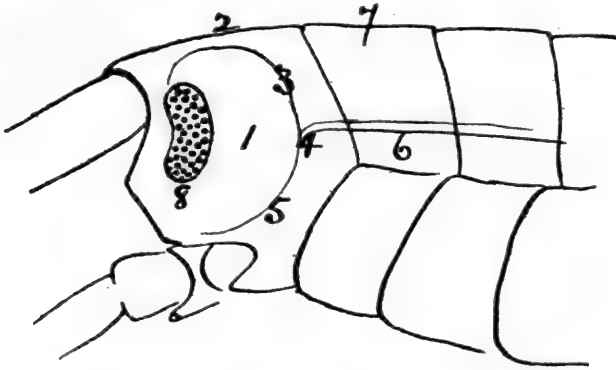


Diagram 1.

The most usual position is at a point on the head behind the eye in the mid-lateral line (1 on diagram). The next position in order of frequency is on the *top* of the head, near the posterior margin, and in this position the white pigment generally takes the form of a large irregular patch (2 on diagram). Then there are three positions (3, 4 and 5 on diagram) along the line of the posterior margin of the brain, an upper (3) and a lower one (5), and one in the mid-lateral line, at the anterior end of the stomach (4). A sixth position which is of great interest is in the mid-lateral line on the 1st peraeon-segment (6). Very occasionally there is a dorsal spot on this same segment (7). The last position calling for special mention is No. 8, immediately below the eye itself. In addition to these positions spots are found occasionally at other points on the head.

This detached white pigment seems also to occupy different situations as regards depth. Speaking very generally streaks and small spots are usually quite superficial, but the larger spots are somewhat deeply embedded in the tissue. The spots 1, 4 and 6 are usually deep; 3 and 5 as a rule deep, 3 being more often so than 5; 2 and 7 are superficial. These constantly recurring positions of the white spots seem suggestive, especially the positions of the dorsal patch and of the spots on the 1st peraeon-segment. One thinks at once, in connection with the dorsal spot, of the nauplius eye of crustaceans, which is known to persist in *Nebalia*. The spot on the 1st peraeon-segment, as well as the two

median-lateral spots on the head (1 and 4), suggest a segmental arrangement. Are we dealing with the loss of factors, which is leading to a more primitive arrangement, giving traces of eyes on two head segments and on the 1st peraeon?

It should be mentioned that the spots recorded were seen in the young when they first emerged from the brood pouch. As a rule the spots remain through life, sometimes changing their shape after a moult (as, e.g. a streak may become a spot, or a patch may break up into several streaks), but very rarely altering in position. Sometimes, but not often, they disappear as the animals get older and become mature. In five instances recorded in our normal stock, four animals hatched with lateral spots, and one, hatched with a dorsal patch, all lost them. In other cases, chiefly among the Albino stock, spots develop with age. There are several records of spots changing their position¹.

In about a dozen out of many thousands of wild *Gammarus chevreuxi* examined similar spots have been found.

The spots appeared almost simultaneously in our different stocks about a year after the "No-white" mutation arose.

1. *The Appearance of Spots in the Pure Red Stock.* This stock consisted of the F_2 Reds from the crosses between Pure Blacks and Pure Reds, separated in January, 1914, and left to interbreed.

When examined on Aug. 26, 1915, all had eyes of the usual bright red colour, except four very large animals, three males and one female. In these the eyes had a very curious appearance, some of the ommatidia being unpigmented; others red, of which some had apparently only part of the retinal cells pigmented, giving the effect of bars or streaks of colour across the cones; while several ommatidia were black or purplish-black (Plate XIV, Fig. 7).

This composition of the eye we now consider to be due to age (cf. ♀ I.E., p. 348), but at the time it was not recognised as such and experiments were carried out to see if it proved inheritable.

The Pure Red stock was examined again on Dec. 6, 1915; 106

¹ 1. An Albino male, hatched with large spot, left side, position 1; at maturity the spot was connected with the eye by a little neck of white pigment.

2. An Albino male, hatched with streak, right side, position 1; two months later, the spot was connected with the eye, a month later still, the spot had completely coalesced with the eye.

3. A Red female, hatched with spot, right side, position 3; at maturity the spot was connected to the eye.

4. An Albino, hatched with many spots; the positions changed with each moult, the only record we have of such an occurrence.

animals were found, amongst them one young one with a large patch of the white pigment spread over the dorsum. *This is the first record of the appearance of spots.* This specimen, a male, became mature in January, 1916, and was mated with six different females, to see if the spots were inheritable, five Red ones from its own stock, one a young one with a similar dorsal patch, and one Hybrid-Black female.

All the matings gave spots in the F_1 generation, the heavy white reticulation appeared in the F_2 and again in the F_3 generations together with the No-white mutation. By May 25, 1916, the male's eyes had become "composite" in appearance (cf. Fig. 7). It died Oct. 25, 1916.

In all, it had 339 offspring, 31 of them spotted, 3 dorsal and 1 both dorsal and on the 1st peraeon-segment: in the F_2 generation, 185 young, 2 with very heavy reticulation, 11 spotted, 2 dorsal: in the F_3 generation, 35 young, 9 spotted, 1 with the heavy reticulation as well, 1 No-white on both sides, and 1 No-white on one side.

The Pure Red stock was again examined on May 5, 1916. 257 animals were found, 252 with the normal bright red eyes, 6 of these with dorsal spots, and 10 with lateral spots; 4 others with very dark pigment in many of the ommatidia, and 1 with the red so dilute as to show as pale pink.

Since then many spotted have been found in this 1914 Red stock. It has now, Nov., 1918, died out.

2. *Appearance of Spots in the Albino Stock.* The spots appeared with the Albino mutation, one of the two original Albino females having a long deep streak of white stretching out from the right eye, which, after a moult, became a large spot, as if the white pigment had all collected at the point farthest from the eye.

The other female (see Allen and Sexton, 1917, Plate VII, Fig. 4) mated with two males, one Pure Red, and one Hybrid Black, and had 99 young, of which four were spotted, two dorsally, and one on the 1st peraeon-segment. (The Hybrid Black male, derived from similar ancestry, evidently carried the factor for spots as well, as, when mated with a wild Pure Black female, it had five spotted young—small lateral spots—out of 87 offspring.)

The spots appeared in all the F_2 matings, the Albinos always giving a much larger proportion than the Coloured.

In the course of experiments designed to determine the laws of inheritance of the colour of the eye, detailed records were kept of all the spots which occurred, and these records made it clear that the

inheritance of spots did not follow the simple Mendelian law. The spotted condition did not behave either as a pure dominant or a pure recessive. Spotted animals mated together always gave both spotted and unspotted offspring. One point, however, came out clearly, namely, that Albinos and coloured strains carrying Albino give a much larger percentage of spotted young than do pure coloured strains.

There is a possibility that the inheritance of spots follows some more complicated Mendelian law, as will be seen from the figures for a certain number of *selected cases* given below. The Mendelian ratios 3:1, 9:7, 15:1 frequently occur in some of the best cases we have, where figures are large, but it is hard to find any consistent theory. The reversal of the proportions given by experiments I.E. and I.E. 30 is curious and may be significant.

3. *Selected Cases.*

Matings of Gammarus chevreuxi; all animals from "spotted" stock.

B=Black, **b**=absence of Black, i.e. Red.

C=Colour, **c**=absence of Colour, i.e. Albino.

Figures for spotted include all spots, patches or streaks of white pigment.

Red carrying Albino × *Red carrying Albino.* Neither spotted.

		Ccbb × Ccbb			
		Unspotted Red	Spotted Red	Unspotted Albino	Spotted Albino
Family I.E.	Expt.	389	132	95	68
	Theory	...	36 : 12	9 : 7	
	Gives	...	385	128	96

Red carrying Albino × *Red carrying Albino* Both spotted.

		Ccbb × Ccbb			
		Unspotted Red	Spotted Red	Unspotted Albino	Spotted Albino
Family I.E.	30. Expt.	83	106	14	50
	Theory	...	7 : 9	1 : 3	
		3		1	
	i.e.	...	21 : 27	4 : 12	
	Gives	...	83	107	16

Red carrying Albino × *Albino carrying Red.* Neither spotted.

		Ccbb × ccbb			
		Unspotted Red	Spotted Red	Unspotted Albino	Spotted Albino
Family Cross 20.	Expt.	100	18	66	38

Red carrying Albino × *Albino carrying Red*. Both spotted.

Ccbb × ccbb

		Unspotted Red	Spotted Red	Unspotted Albino	Spotted Albino
Family Cross 50.	Expt.	123	58	84	116
Theory. <i>Unspotted Albino</i> : <i>Spotted Albino</i> is nearly 7 : 9 which would require 87 : 112.					

Black carrying Albino × *Black carrying Albino*. Neither spotted.

CcBB × CcBB

		Unspotted Black	Spotted Black	Unspotted Albino	Spotted Albino
Total Cases.	Expt.	166	12	54	20
Theory		15	: 1	3	: 1
		3		1	
i.e.	...	45	: 3	: 12	: 4
Gives	...	180	12	48	16

Black carrying Albino × *Black carrying Red and Albino*. Neither spotted.

CcBB × CcBb

		Unspotted Black	Spotted Black	Unspotted Albino	Spotted Albino
Total Cases.	Expt.	145	7	31	13
Theory as above		45	: 3	: 12	: 4
Gives	...	135	9	36	12

Black carrying Albino × *Red carrying Albino*. Neither spotted.

CcBB × Ccbb

		Unspotted Black	Spotted Black	Unspotted Albino	Spotted Albino
Family Cross 3.	Expt.	305	24	71	29
Theory as above		45	: 3	: 12	: 4
Gives	...	301	20	80	27

Black carrying Red and Albino × *Red carrying Albino*. Neither spotted.

CcBb × Ccbb

		Unspotted Black	Spotted Black	Unspotted Red	Spotted Red	Unspotted Albino	Spotted Albino
Total all cases		140	7	110	9	78	19

That is:		Unspotted Coloured	Spotted Coloured	Unspotted Albino	Spotted Albino
Expt.	...	250	16	78	19
Theory as above		45	: 3	: 12	: 4
Gives	...	255	17	68	23

Albino × *Albino* (both may carry *Red* and *Black* or only one of them).

Both spotted.

		Unspotted Albino	Spotted Albino
Family C.	17 b. Expt.	143	97
	Theory... ..	9	: 7
	Gives	135	105

Albino × *Albino* (may carry both *Red* and *Black*). One unspotted,
one spotted.

		Unspotted Albino	Spotted Albino
Family A.	4 b. Expt.	143	98
	Theory... ..	9	: 7
	Gives	135	105

4. Attempts to establish Pure Stocks.

In consequence of the results just recorded attempts were made to establish by breeding, pure strains (A) of normal unspotted animals and (B) of spotted animals, and to this end two sets of experiments were started. Success did not attend our efforts, no pure strains being obtained, but as the experiments show several points of interest the details are summarised below.

A. Experiments to establish an Unspotted strain.

A Hybrid Black stock was selected (i.e. Black carrying Red) which had never been crossed with Albino, and had given very few spots, and out of this a number of unspotted pairs were taken.

When comparing the relative proportions of spotted animals already given under the heading "selected cases" with those derived from the experiments now to be recorded, it must be remembered that the former are from animals belonging to a very spotted stock carrying Albino, whilst the latter are from a stock which throws very few spotted and carries no Albino.

The unspotted pairs. 16 pairs of normal-eyed unspotted animals were mated. The detailed records of all the experiments are kept in the laboratory at Plymouth for reference, and only the figures of the largest families are given here.

(a) Pure Red male × Pure Black female; 1 pair.

(b) Hybrid Black male × Hybrid Black female; 3 pairs.

One family gave 329 unspotted, and 19 spotted coloured young; a percentage of 5.4 spotted.

Another family gave 346 unspotted and 22 spotted; a percentage of 6.

The third family consisted of 57 unspotted.

- (c) Hybrid Black male
- \times
- Pure Red female; 5 pairs.

One family (Exp. IX) gave 228 unspotted and 50 spotted; a percentage of 18.

- (d) Pure Red male
- \times
- Hybrid Black female; 2 pairs.

One family (Exp. X) gave 439 unspotted and 8 spotted; a percentage of 1.8.

The other gave 123 unspotted and 16 spotted; a percentage of 11.5.

- (e) Pure Red male
- \times
- Pure Red female; 5 pairs.

One family gave 148 unspotted, and 7 spotted; a percentage of 4.5.

Another (Exp. V) gave 173 " " 9 " " " 4.9.

Another gave 281 " " 13 " " " 4.4.

Another (Exp. IX b) gave 76 " " 6 " " " 7.3.

In Experiment IX *b* the Red male (from *d*) which had given the lowest percentage of spotted, 1.8%, was mated the Red female (from *c*) which had given the highest, 18%.

For the F_2 generation, we mated the F_1 young Reds to see (1) if the homozygous for colour would give any different results from the heterozygous, and (2) if a mating giving a high percentage of spotted in the F_1 generation would continue to give it in the F_2 .

The Experiment IX, which had given the highest percentage, 18%, was taken and nine matings of F_1 unspotted Reds were made; results as follows:—

One family gave	306 unspotted Red and 28 spotted; a percentage of 8.3.
Another family gave	287 " " 5 " " " 1.7.
Another " "	253 " " 6 " " " 2.3.
Another " "	150 " " 4 " " " 2.6.
Another " "	164 " " 10 " " " 5.7.

The percentage of spotted offspring from each of the nine pairs was low, varying from 8.3% to 0 or 1%.

This result may be compared with the F_1 matings of unspotted Reds from the Experiment X¹, which had given the lowest percentage, 1.8%. The percentage varied from 4.8% to 0%. The largest family gave 426 unspotted and 6 spotted, a percentage of 1.4.

It may also be compared with the F_1 matings from Experiment V (from *e*) which gave a mean percentage 4.9.

One pair gave	137 unspotted and 5 spotted; a percentage of 3.5.
Another pair (1) gave	146 " 5 " " " 3.3.
Another pair (2) " "	126 " 23 " " " 15.4.

¹ The largest families from the heterozygous matings of the F_1 young from this same experiment were:

Hybrid \times Red	One family of 657 unspotted and 11 spotted; a percentage of 1.6.
Hybrid \times Hybrid	" " 104 " " 3 " " 2.8.

These last two pairs were then crossmated, the results obtained were: from the male of pair 1 mated with the female of pair 2 there were 81 unspotted offspring and 16 spotted, a percentage of 16.5. From the other pair (male of (2) and female of (1)) there were 129 unspotted and 7 spotted young, a percentage of 5.1.

An experiment from the family of the (b) group, which gave no spots in the F_1 generation, is interesting. Two F_1 young Reds were mated and had 180 young before the first spot appeared. In all, there were 275 unspotted and 6 spotted, i.e. 2.1%, but the other 5 spotted all came in the last brood, the male dying before the eggs were hatched.

The Spotted Pairs. For comparison with these unspotted pairs a number of spotted pairs having the same origin were mated. Only 6 spotted adult animals could be found in the stock; their spots were all *small and lateral*. The percentage of spotted offspring was, on the whole, less than in the matings of the unspotted. One family gave 176 unspotted and 7 spotted, a percentage of 3.8, and another gave 88 all unspotted.

It has been found impossible to get a Pure Unspotted Stock. A mating of Unspotted \times Unspotted gave 57 unspotted young in the F_1 generation, but the spots appeared in the next generation.

Another mating, Spotted \times Spotted, is remarkable from the fact that the spotted animals gave 88 all unspotted young. The spots, however, reappeared in the F_2 generation, the principal types of spots being represented, dorsal, lateral, and on the 1st pereon-segment.

The lowest percentage of Spotted in the F_2 generation was obtained from the 1.8%, Experiment X. The Unspotted young were mated together, but the spots appeared again in the percentage of 1.6 spotted. Taking all the offspring of the Unspotted F_1 matings in this experiment, 1617 were hatched, of which 27 were spotted.

In the Experiment IX, which gave the highest percentage in the F_1 generation, 18%, the Unspotted F_1 were mated together and 1863 young were produced, of which 71 were spotted, i.e. a percentage of only 3.8 spotted.

An attempt was made to mate the Spotted F_2 young from the different experiments, to find what proportion of spotted would be given. Unfortunately many died, and only two or three large families could be obtained, not enough to base conclusions on.

It would seem, however, that animals with large dorsal spots give the largest number of spotted young. One pair, both spotted dorsally,

from Experiment IX, had 332 unspotted young and 41 spotted (i.e. 11% spotted). Two of their young F_2 , also both spotted dorsally, had one brood of 12, 8 of which were spotted.

In another Experiment, an F_2 male mated with two females, all dorsally spotted, had with the first female 170 unspotted and 17 spotted, i.e. 9%, and with the other 41 unspotted and 11 spotted.

Summary. In the Coloured Spotted strain experimented on, when unspotted animals were mated together, families were obtained in which the percentage of spotted young varies from 0% up to 18%. When spotted animals were mated a similar result was obtained and the percentage of spotted was not increased.

If the percentages of spotted for the different families in these and other experiments of a similar kind are arranged in order (taking only those families composed of 50 or more individuals) they form a fairly continuous series from 0 to 18% of spotted.

The eye-colour of the parents, i.e. whether the eyes are Black or Red or Hybrid Black (black carrying red) makes no difference in the percentage of spotted offspring.

B. *Experiments to establish a Pure Spotted Strain.*

The Albinos always give a much larger proportion of spotted than the Coloured Stocks¹, so it was decided to try to establish a Pure Spotted Strain amongst them. For this purpose a family (C.17b)² was taken, which had given a very high percentage of spotted through several generations (cf. p. 358).

As the Albinos carry either Pure Black or Pure Red or both Black and Red which do not show unless mated with Coloured, and as we did

¹ When Albinos are mated with Coloured carrying Albino, the Albino offspring always give a much higher percentage of spotted than do the Coloured offspring, as the following instances show:

1. Albino \times Colour carrying Albino, neither spotted: F_1 young, 44 unspotted and 2 spotted Coloured; 28 unspotted and 9 spotted Albino: percentage of spotted Coloured 4.3%, of spotted Albino 24.3%.

2. Albino \times Colour carrying Albino, neither spotted: F_1 young, 76 unspotted and 1 spotted Coloured; 56 unspotted and 13 spotted Albino: percentage of spotted Coloured 1.3%, of spotted Albino 18.8%.

3. Albino \times Colour carrying Albino, one spotted: F_1 young, 85 unspotted and 14 spotted Coloured; 41 unspotted and 29 spotted Albino: percentage of spotted Coloured 14.1%, of spotted Albino 41.4%.

² This family was descended from a pair of spotted Albinos, the male being VI. C. 2r and the female I. G. 1c, shown on Plates I and II, Allen and Sexton, 1917.

not know if being homozygous or heterozygous for colour would cause a difference in the results, we had first of all to test the animals with Pure Red mates before mating Albino with Albino.

The experiments therefore fall into two divisions:—

1. Albinos mated with Reds; and
2. Tested Albinos mated with tested Albinos.

1. *Albinos mated with Reds.*

The Albinos were taken from different broods of the C. 17 *b* family; the Reds were drawn from one stock as far as possible, a mixed stock (Pure Black \times Pure Red) with no admixture of Albino and with very few spots, the same stock as that from which the animals for the Unspotted strain experiments were taken.

A great many were tested, but only the details of the larger families are given. The Red mates were all unspotted.

The following are the results of matings between the Unspotted Albinos and Pure Reds:—

1.	Male	carrying Red	67 unspotted offspring	10 spotted
2.	Female	„ Black and Red	98 „ „	5 „
3.	Female	„ „ „	33 „ „	10 „
5.	Female	„ Black	27 „ „	8 „
9.	Male	„ Black and Red	24 „ „	3 „
10.	Female	„ Red	32 „ „	6 „
11.	Male	„ „	31 „ „	2 „
12.	Male	„ „	51 „ „	2 „
13.	Male	„ „	56 „ „	8 „
14.	Male	„ „	66 „ „	3 „
64.	Female	„ Black and Red	50 „ „	11 „
65.	Female	„ „ „	54 „ „	5 „

The Albinos, numbered from 1 to 14, are all from one brood.

The following are the results of matings between the Spotted Albinos and Pure Reds:—

20.	Male	carrying Black	63 unspotted young and 1 spotted
77.	Female	„ Black and Red	51 „ „ 2 „
78.	Female	„ „ „	10 „ „ 1 „
80.	Male	„ Black	39 „ „ 3 „
82.	Male	„ „	69 „ „ 5 „
96.	Male	„ „	105 „ „ 6 „
97.	Female	„ Black and Red	29 „ „ 11 „

2. *Tested Albinos mated with tested Albinos.*

Unfortunately a large number of the tested Albinos died before they could be used in this section. The results of the survivors are as follows:—*The unspotted pairs*—

Expt. III*b*. ♂ 9 × ♀ 3, both carrying Black and Red; 155 unspotted young and 118 spotted; 43·2% spotted, or 9:7 ratio.

Expt. X*b*. ♂ 1 and ♀ 10, both carrying Red; 7 unspotted and 11 spotted.

Expt. X*c*. The same male, which had now developed spots, and ♀ 64; 28 unspotted and 57 spotted; 67% spotted.

Expt. XI*b*. ♂ 11 and ♀ 65, one carrying Red and one Black and Red; 7 unspotted and 5 spotted.

Expt. XII*b*. ♂ 12 and ♀ 78, both carrying Black and Red; 13 unspotted and 17 spotted.

Two pairs of unspotted F_1 young from Expt. X*c* were mated; one pair gave 26 unspotted and 68 spotted; the other pair 12 unspotted and 20 spotted.

♀ 65 from XI*b* was also put with ♂ 82, a spotted Albino carrying Black, and gave 21 unspotted and 15 spotted.

The spotted pairs. Of the spotted F_1 young of Expt. III*b* one male was mated with three females; from the first there were 41 unspotted and 53 spotted young; from the second 17 unspotted and 42 spotted; and from the third 8 unspotted and 51 spotted.

Expt. 116*b*. (From Family I.E., see pp. 348 and 354.) Male and female both carrying Red; 17 unspotted, 30 spotted.

Expt. 93*d*. (From I.E.) Male and female both carrying Red; 16 unspotted, 17 spotted.

Two other experiments may be quoted: a male carrying Red was mated with two females from different broods of Family C. 17*b* (these were not tested for colour); one gave 31 unspotted young and 30 spotted; the other 33 unspotted and 32 spotted.

Summary. When Albinos were mated with Pure Reds from a strain which only very occasionally threw spotted animals, the percentage of spotted in a family varied from 1 or 2% to 18%, a result which does not differ from that obtained when coloured animals from a spotted strain were mated together (cf. p. 357).

The percentage of spotted did not seem to be influenced by the colour factor carried by the Albino parent, and there is no obvious

difference in the percentage of spotted young in families whose Albino parent was spotted and in those in which the Albino parent was unspotted.

When Albino was mated with Albino the percentage of spotted young in the families was very much higher, from 43 % to 86.4 %. There seems no significant difference in the percentage between families of spotted and unspotted parents.

A pure spotted strain was never obtained.

V. SPOTS WITH THE NO-WHITE MUTATION.

As already explained the No-whites appear to be of two different kinds, (a) those which we call Pure No-whites, which arise suddenly and independently in the different stocks, and (b) those which possess at least the normal quantity of white pigment at the time of hatching, but gradually lose it with growth, until they eventually become completely No-white.

(a) *Pure No-whites.* Many of the animals with pure No-white eyes, arising suddenly from normal parents, are found to carry spots of white pigment on the head and 1st segment similar to those described in the former section.

Two large families in which this occurred may be quoted here, one from a pair of Black without any admixture of Albino, and the other from a Red pair carrying Albino. (Family I.E.)

In the first instance, 1022 young were produced in three generations, of which 70 had either both eyes or one eye No-white; of the 47 Pure No-white, 32 were unspotted and 15 spotted, that is about a third spotted; of the 23 one-sided No-whites, 11 were unspotted and 12 spotted, that is a half spotted.

In the other instance the proportions of spotted to unspotted were quite different. 780 young were produced in one generation, 6 Pure No-whites and 21 one-sided amongst them. Of the 6 Pure No-whites 2 were unspotted and 4 spotted; whilst of the one-sided 2 were unspotted and 19 spotted.

Taking all the records from all sources together and summing them up shortly there were 32 spotted Pure No-whites; of these 4 had large spots or patches on the right side only, 10 on the left side only, 3 on both sides, 2 dorsal, 8 on the 1st peraeon-segment, 4 both lateral and on peraeon-segment, and 1 lateral, dorsal and on peraeon-segment.

Whenever two unspotted No-whites of this kind have been mated

together all the offspring have been invariably unspotted No-whites. This applies to all such matings which amount to many hundreds—to Coloured No-whites, both those pure for Colour and those carrying Albino, and to Albino No-whites whether mated together or with Coloured No-whites.

We have never succeeded in rearing two spotted No-whites to maturity at the same time, so that it has not been possible to mate them.

We also obtained a number of animals in which one eye only was No-white, which also carried spots, generally on the same side as the No-white eye. The spots were very large, usually very deep in the tissue, and nearly always in the same definite positions (4, 6 and 2 on the diagram, p. 353)¹.

Altogether 59 spotted one-sided No-whites appeared in the different strains; of these 15 had lateral spots, i.e. position 4 on the same side as the No-white eye; 8 on the same side lateral and dorsal; 4 on the 1st peraeon-segment on the same side; 3 on the same side, lateral and peraeon-segment; 6 on the same side, lateral, dorsal and peraeon-segment; 2 on peraeon-segment same side and dorsal; 7 on both sides, lateral; 1 dorsal; 6 on the opposite side lateral; 4 on the peraeon-segment same side and opposite side lateral; 2 on the peraeon-segment opposite side; and 1 on opposite side lateral and dorsal. When the two eyes are different therefore in respect of No-white, spots tend to be more frequent on the No-white side; a point of interest when it is remembered that unspotted pure No-whites have never been known to give spotted offspring.

Matings in which one parent is an Albino spotted dorsally and the other a No-white are of special interest on account of the high percentages of spotted offspring.

An instance may be given of one Coloured No-white male mated with three Albino females, descendants of C. 17 *b*, all with large dorsal spots, the third having a spot also, left side, position 5.

With female 1, which carried Black and Red, the offspring produced were 29 normal-eyed unspotted, 23 normal spotted and 1 spotted No-white; a percentage of 45·3 spotted.

With female 2, which carried Black, there were 75 normal unspotted and 31 spotted (1 No-white); a percentage of 28·9 spotted.

With female 3, which carried Black, there were 32 normal unspotted and 10 spotted; a percentage of 23·8 spotted.

¹ In the next paragraph spots in position 4 are described as "lateral," in position 6 as "peraeon-segment," and in position 2 as "dorsal."

Matings in which one parent is a normal-eyed Coloured spotted dorsally and the other a Coloured No-white give a much lower percentage of spotted. For instance the coloured No-white male just quoted was mated with an F_1 normal-eyed Red from the first female's offspring. The number of young was 46 normal-eyed (39 unspotted and 7 spotted) and 47 No-whites, i.e. a percentage of 15 spotted amongst the normals.

Another mating of the same kind gave 77 unspotted Black and 10 spotted, 77 unspotted Red and 1 spotted; a percentage of 6.7 spotted.

(b). *Animals which become No-white as they grow older.* The matings of these animals are remarkable for the fact that considerably more than half the young hatched are spotted (140 unspotted to 180 spotted and 1 one-sided No-white), with the spots usually large and several of them with more than the normal quantity of white pigment in the eyes. If two such animals are mated together when young, i.e. when some white pigment is present in the eyes, all the offspring are normal. We have not yet had ready to mate at the same time a male and a female in which all the white pigment had disappeared owing to age, so that we can make no statement from actual observation as to whether or not the offspring of such a pair would have white pigment.

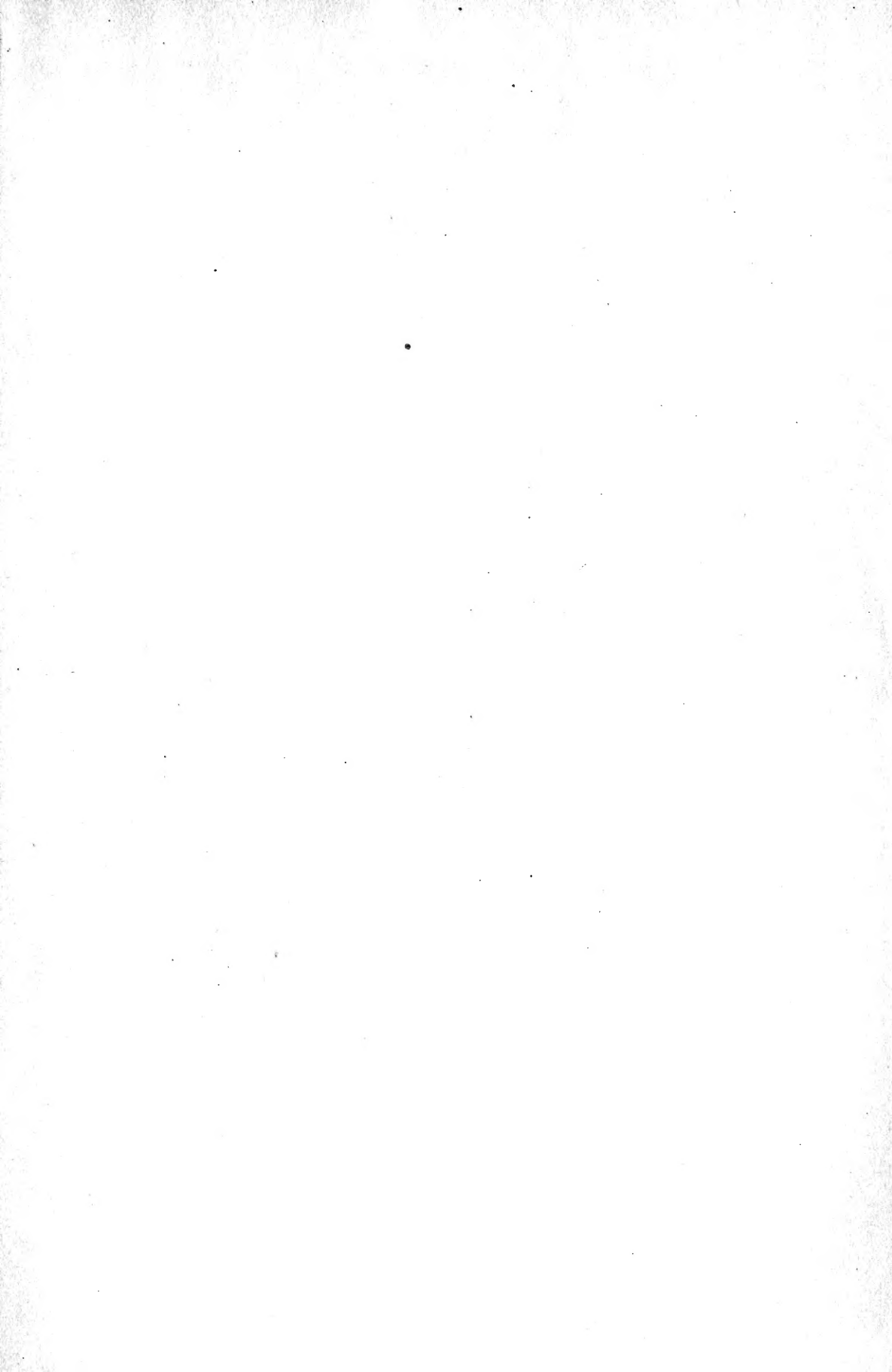
The details of all the experiments are preserved at Plymouth. Our best thanks are due to Miss A. R. Clark for valuable help in the care of the broods and in the examination of the young for eye-colour and the presence of spotting.

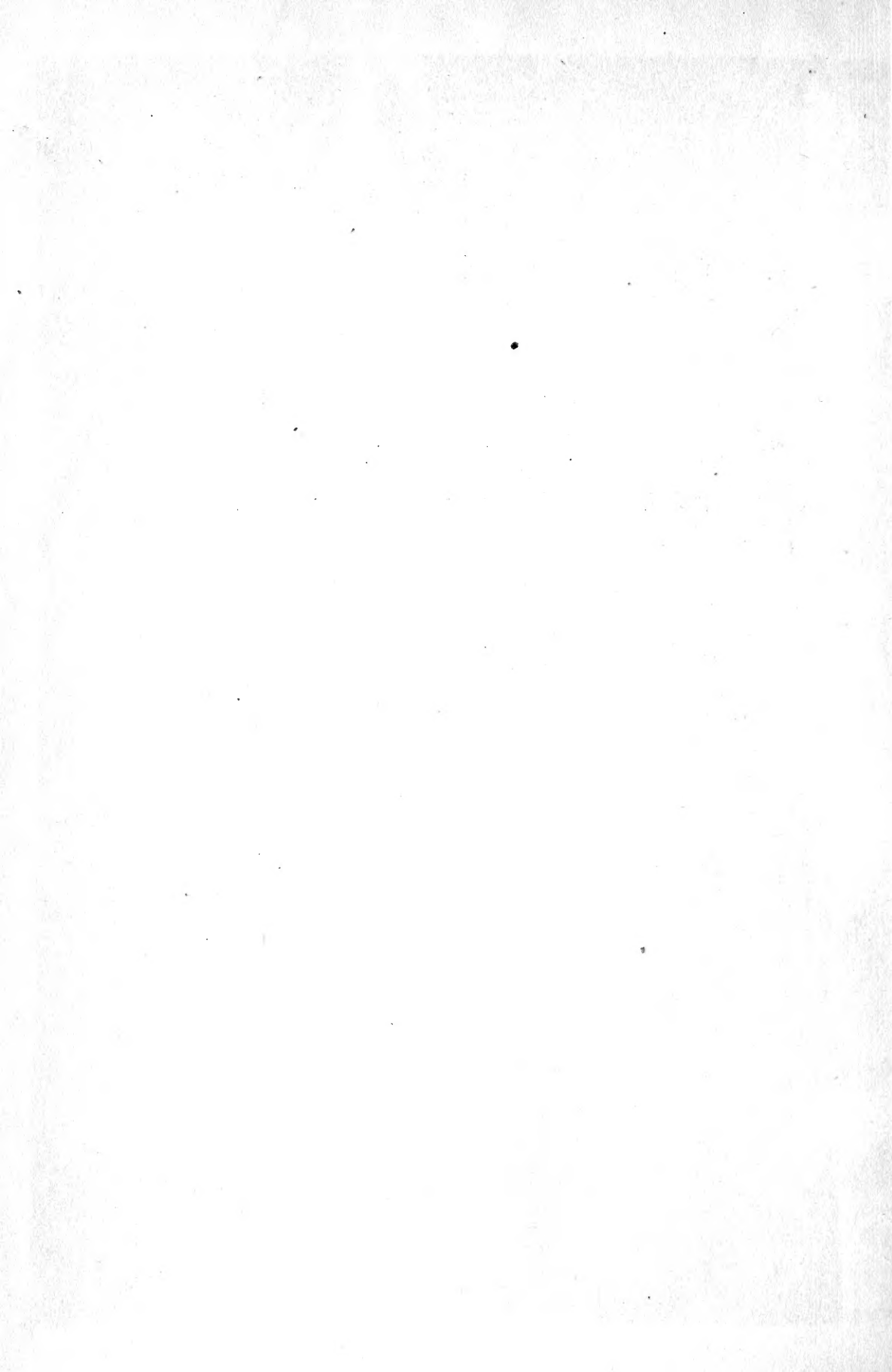
EXPLANATION OF PLATE XIV.

Gammarus chevreuxi. All figures $\times 58$.

- Fig. 1. Normal Black Eye (B) ♀. Age $4\frac{1}{2}$ months. Figured a few hours before moulting.
- Fig. 2. Red eye darkened with age. ♀ I. E. (see p. 348). The drawing was made 18 months after the animal was hatched and after she had extruded her 24th brood. Up to about a month of that time the eyes had been bright red. She laid four batches of eggs after the drawing was made and was then eaten by a second mate.
- Fig. 3. Normal Albino Eye (A + B + R) ♀. Age $5\frac{1}{2}$ months.
- Fig. 4. Red, No-white Eye (RN + A). Young ♂.
- Fig. 5. Colourless (Albino, No-white) Eye. (AN + R) ♀. Age $9\frac{1}{2}$ months.
- Fig. 6. Head of Red-eyed male from Pure Red Stock. (R) showing (a) dorsal patch, (b) small lateral spot position 1, and (c) anterior end of the stomach.
- Fig. 7. "Composite" coloured eye, referred to on p. 354. The eye was figured about 4 months before the animal, which was very old, died.







QH Journal of genetics
431
AlJ64
v.9
cop.2
Biological
& Medical
Serials

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY

